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THE UNIVERSITY OF ALBERTA

PHYTOCHEMICAL INVESTIGATION OF *OPLOPANAX HORRIDUM*

by

AMAL RUSHDY

A THESIS

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Phytochemical Investigation of Oplopanax horridum" submitted by Awni Nashy in partial fulfillment of the requirements for the degree of Master of Science.

Supervisor

Date February 27, 1979

TO HASSAN, AMGAD, ASHRAF,

AND MOTHER

TO HASSAN, ANGAD, ASHRAF,

AND MOTHER

ABSTRACT

Oplopanax horridum Miq. (Devil's Club), a plant of the Araliaceae Family, was re-evaluated biologically for hypoglycemic activity.

Aqueous extracts, using different experimental conditions, were tested and no hypoglycemic effect was observed.

The volatile oil of the same plant (1.37% of dry weight), prepared by steam distillation, was analyzed by the use of gas-liquid chromatography, infrared spectrophotometry, ultraviolet spectroscopy, nuclear magnetic resonance and mass spectrometry. It was found to contain the following compounds in order of elution from a Lac-728 column: diethyl ether (1.24%), ethanol (24.99%), mixture of n-butyraldehyde and hexadiene (0.93%), two isomeric unsaturated alcohols, $C_{10}H_{20}O$, (0.75%, 0.97%), a homologous series, probably of acetals and esters, $C_7H_{14}O_2$ (1.74%), $C_8H_{16}O_2$ (trace) and $C_9H_{18}O_2$ (0.56%), an octanal (1.64%), probably a methyl ester, $C_9H_{18}O_2$, (0.63%), 2-nonanone (1.17%), and probably isoamyl acetate (0.78%).

The volatile oil was also found to contain four sesquiterpene hydrocarbons of the formula $C_{15}H_{24}$ (12.77%), and three oxygenated sesquiterpenes (12.11%): $C_{15}H_{24}O$, $C_{15}H_{24}O_2$ and $C_{15}H_{26}O_2$.

Two of the sesquiterpene hydrocarbons may belong to the cadinane type sesquiterpene.

The volatile oil prepared by steam distillation did not contain oplopanone.

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I. INTRODUCTION

This thesis is written in two parts. Part I describes a reinvestigation of extracts of Oplopanax horridum (Devil's Club) root bark, which has been reported to have oral hypoglycemic activity (1). Part II is an investigation of the volatile oil contained in Devil's Club root bark.

The purpose of this study was to find a useful drug for the treatment of diabetes mellitus by oral administration.

II. LITERATURE SURVEY

1. Diabetes

The fascinating history of diabetes can be traced back to the oldest medical text existing, a papyrus written about 1500 B.C. The name "diabetes" was first given to the disease by Aretaeus, 135 A.D. (2). Clinical accounts of the disease were given by Japanese and Chinese physicians of the third century, the Indian doctor Susruta of the fifth century, and the Arabian physicians Rhazes and Avicenna (3) in the ninth and tenth centuries. In the middle of the seventeenth century Thomas Willis, an English physician, studied the disease and diabetes mellitus was distinguished from diabetes insipidus (2).

The history of diabetes seems continually to go through stages of clarification and complication. Until the end of the eighteenth century, the disease was almost inexplicable. Through the observation of von Mering and Minkowski in 1889, it became recognized that changes in the pancreas could cause diabetes (4). Then Langerhans described his islets, and the name "insulin" was given to the hypothetical active substance (3). Insulin was isolated by Banting and Best in 1922 (2) at the University of Toronto.

Diabetes seemed, for awhile, to be due to the simple failure of the beta cells to secrete insulin. By Houssays' demonstration of the effect of experimental hypophysectomy on glucose metabolism (3), and the production of diabetes by administration of growth hormone, in both animals and humans (5, 6), it became obvious that diabetes could not be regarded

as being always due to simple failure of the beta cells of islets of Langerhans (7).

At present it is thought that the primary defect lies outside the pancreas and that the islet tissue is secondarily damaged with eventual loss of the ability of producing insulin (8).

A study of the prevalence of diabetes in: the Americas (9, 10, 11), western Europe (12), Asia and Australasia (13), eastern Europe (14) and Africa (15, 16), indicates that the disease is a major medical and public health problem. Although much is known about the biochemical basis of diabetes, too little is known to give an accurate definition of the disease. Some of the characteristic symptoms of diabetes are: hyperglycemia, glucosuria, polyuria, polydipsia, and possible loss of weight and strength. Ketone bodies (acetoacetic acid, acetone and β-hydroxybutyric acid) may be detected in the blood and urine of diabetic subjects (2). All these metabolic effects of diabetes are due to a lack of effective insulin; the reason for this lack is not completely known (17, 18).

Diabetes can be classified into two main types: the growth-onset "juvenile", "brittle" type, and the maturity-onset "adult", "stable" type (19, 20).

The reported effects of insulin in vivo and in vitro are bewildering and numerous. Insulin has a profound effect on the metabolism not only of carbohydrates but also of fats and proteins (2, 21).

2. Hypoglycemic Agents

In screening large numbers of chemicals for preliminary activity, it is of prime importance to select a physiologic end point. As an ideal antidiabetic agent is one which can normalize all the metabolic derangements occurring in diabetic patients, there will be many physiologic end points, but many of these are not easy to determine. Historically attention has been directed to glucose metabolism (22). Changes in blood glucose concentration are chosen as a tool in screening for antidiabetic agents because of convenience (22, 23). The term hypoglycemic, in most instances, is applied to drugs used in diabetes but it is obvious that antidiabetic drugs are hypoglycemic agents, and the contrary is not always true (24).

(a) Insulin

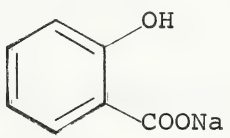
Insulin is the only hypoglycemic agent which can restore the deranged metabolic process of diabetes (25). It was obtained in the first therapeutically useful form by Banting and Best in 1922, and was isolated in the crystalline form in 1926 (26). As crystalline insulin had an extremely short duration of action (27), longer acting preparations were obtained by combining it with higher molecular weight proteins such as protamine or globin (28) or histone (29). Further work in this field resulted in the discovery of Lente insulins (30, 31) in which the duration of action could be controlled by giving special consideration to the concentration of zinc and type of buffer.

Although insulin is considered to be the best available therapeutic agent for diabetes, it is not the ideal drug because of its several side effects (25). Being a protein, insulin is not effective orally (31) and must be given parenterally which is an inconvenience (25). It is also capable of producing allergic reaction in sensitive individuals (29). As there is no standard dosage for insulin, each case must be studied individually and cautiously to avoid dangerous hypoglycemic convulsions which can result from overdosage (27, 31). Even with good control of diabetes with insulin, the development of diabetic complications can not always be prevented (25). Because of these numerous disadvantages of insulin, a great deal of research has been carried out to produce an insulin substitute which does not have these drawbacks (2).

(b) Oral Synthetic Hypoglycemic Agents

The Salicylates:

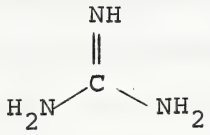
Reports on the hypoglycemic effects of sodium salicylate (I) were published in 1867-1877. Salicylates were not used clinically as hypoglycemic agents because of their side effects at the effective large doses (2).



I

Guanidine:

The first reports on the hypoglycemic effect of guanidine (II) were in 1918. Guanidine was never widely used

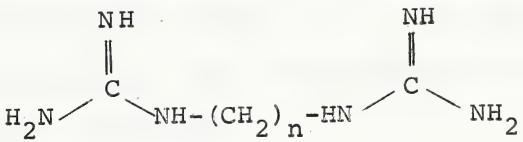


II

because of its high toxicity. But it served as a pattern for newer hypoglycemics with less toxicity (2).

The Diguanidines:

These compounds (III) may be regarded as two guanidine molecules joined by a series of methylene groups. It was noticed that the maximum hypoglycemic activity was reached when

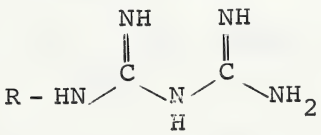


III

the number of methylene groups was ten. This compound known as synthalin A was introduced into diabetes therapy in 1920's. Synthalin B, the dodecamethylene derivative, was less active (2). Because of the tendency to cause kidney and liver damage the use of these two compounds as hypoglycemics was discontinued (31).

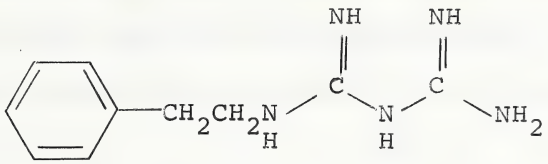
The Biguanides:

The basic structural formula of these compounds is IV. As the result of further attempts to produce useful compounds,



IV

a series of biguanides were introduced in 1929. Since that time little work was done in the area until 1957 when the hypoglycemic activity of the newly synthesized phenformin (V) was reported (2).

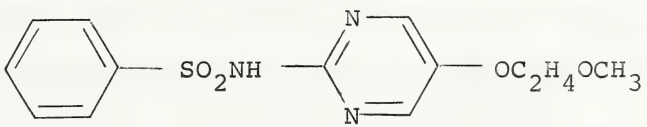


V

The exact mechanism by which biguanides lower the blood sugar is not yet fully understood (32, 33). Steiner and Williams (34) have proposed that these compounds might act by stimulating the anaerobic glycolysis of glucose (35), through the inhibition of certain enzyme systems of the mitochondria which are involved in Krebs cycle oxidations.

The Sulfonamides:

Savagone (36) was the first to publish in 1941 that certain sulfonamides lower blood sugar in man. However, little research was done on sulfonamides as hypoglycemics until the advent of sulfonylureas in 1950's (2). After nine years of study on 600 sulfonamide derivatives a new class of hypoglycemic sulfonamides was discovered (24). Glymidine (VI), one of these derivatives, was introduced in 1964.

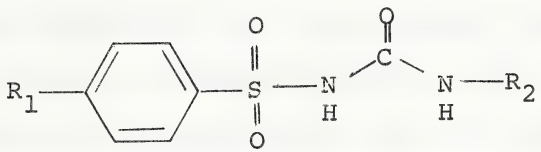


VI

It is believed to act by stimulating the beta cells to secrete insulin.

The Sulfonylureas:

The sulfonylurea drugs have been systematically studied and were first used in diabetic patients in 1955 (37, 31). Chemically they have the general structural formula VII.



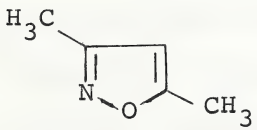
VII

As a result of the extensive laboratory research work done by Loubatières et al. (38) since 1944-1967, it is believed that sulfonylurea derivatives stimulate the beta cells of islets of Langerhans and liberate an increased quantity of endogenous insulin into the blood.

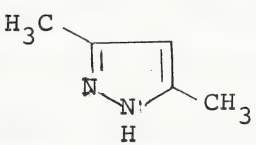
The era of oral agents had helped the improved treatment of countless diabetic patients (31), who would have been treated inadequately by diet alone because of failure of acceptance of daily injections of insulin (39). Although these oral hypoglycemics were received enthusiastically by the medical profession (40), they should not be considered as orally given insulin or substitutes for insulin (41). The proper selection of diabetic patient is an important factor in effective therapy. Beside this limitation, they are contra-indicated in many cases (41, 42, 43), and have several side reactions and toxic effects (43, 44, 45).

The Pyrazoles and Isoxazoles:

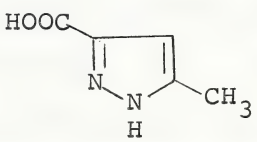
Disadvantages of sulfonylureas and biguanides have stimulated a continuing search for other orally effective hypoglycemic agents. Dulin and Gerritsen were the first to report in 1963 that 3,5-dimethylisoxazole (VIII) is a potent oral hypoglycemic agent (2). It was also reported that 3,5-dimethylpyrazole (IX) was a potent hypoglycemic agent (46, 47), which was metabolized to a more potent compound, 5-methylpyrazole-3-carboxylic acid (X)(48).



VIII



IX



X

Although the mechanism of action of these compounds is still not understood, it is believed to be different from that of insulin, sulfonylureas or biguanides (46). They affect both carbohydrate and fat metabolism (49).

(c) Hypoglycemic Agents from Plants

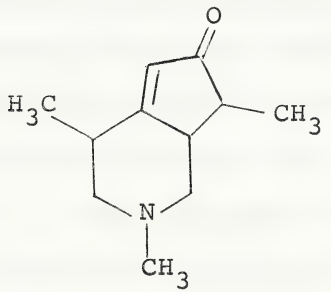
The importance of plant derived medicinals is often underestimated in modern medicine. Such compounds as digitoxin, quinine, quinidine, reserpine, ergotamine, morphine, codeine, papaverine, atropine, scopolamine, protoveratrine A and B, ephedrine, pilocarpine, physostigmine, caffeine and colchicine are only a few to mention presenting a broad range of pharmacologic activities. Natural products have been and still remain an important source of biologically active substances (50).

The belief that hypoglycemic agents derived from plant origin may prove to be useful in the treatment of diabetes, like Rauwolfia in the treatment of hypertension, has led to an intensive search for drugs in ancient medicine (23). The awakened interest in the old materia medica resulted in testing a variety of extracts of plant species belonging to different genera which were mentioned in literature (23). The common procedures, which have been chosen for that research, were the hypoglycemic responses in normal and alloxan diabetic animals. In some cases the improvement in oral glucose tolerance test has been observed, as well as the effect on the acute hyperglycemia of anterior pituitary-treated animals (23). The classical investigation, carried out by Chopra and his colleagues (51, 52, 53), threw much doubt on the usefulness of plant remedies and the field fell into disrepute until recently.

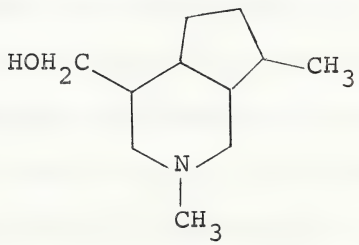
Svoboda et al. (54) have demonstrated that six alkaloids isolated from Catharanthus roseus; catharanthine hydrochloride, leurine sulfate, lochnerine, tetrahydroalstonine, vindoline and vindolinine dihydrochloride, were found to produce varying degrees of blood sugar lowering, not less than that of tolbutamide. The leaves of C. roseus were reputed as folk remedy for diabetes, in the form of tea, by the people of South Africa, Natal, Australia, South Vietnam, the Philippines and England. Several studies have failed to confirm this action by using the crude extract (55). However this folkloric reputation led to its intensive phytochemical investigation

which resulted in the discovery of the effective oncolytic alkaloids (56), vincristine and vinblastine (57).

The dried leaves of Tecoma species have been used by natives of Mexico as an oral antidiabetic remedy in the form of simple infusion or fluid extract (58). The antidiabetic action was not apparent in phloridzin-induced diabetes in laboratory animals in 1899, but clinical tests on diabetic patients gave favorable results (59). Hammouda et al. (60) reported the isolation of tecomine (XI) and tecostamine (XII), two alkaloids from the leaves of Tecoma stans. They reported that these two alkaloids have potent hypoglycemic action when injected intravenously. Similarly, vincamine, the major alkaloid of Vinca minor, has been reported to lower blood sugar when administered i.v. (50).

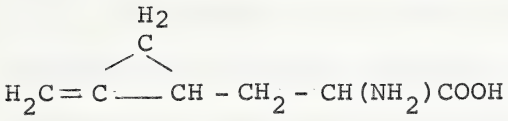


XI

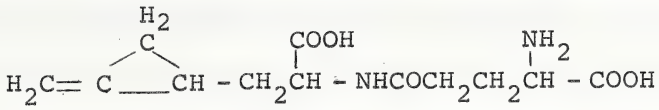


XII

Two amino acids of unusual structure (hypoglycin A, XIII, and hypoglycin B, XIV) were isolated from the seeds of Blighia sapida (61). They produce fatal hypoglycemia in laboratory animals. Hypoglycin A was found to be effective in alloxan diabetic rats (50).



XIII



XIV

Garcia (62) reported that decoction of Lagerstroemia speciosa leaves (banaba), which is used as a popular folkloric remedy for diabetes in Philippines, had a blood sugar lowering effect in normal rabbits. Plantisul tablets prepared from the same plant have been reported successful to some degree in diabetic patients (63, 64).

The Russian workers, Karaev et al. (65), reported that extracts of Cichorium intybus, Lactuca sativa, Coriandrum sativum, Aloe arborescens and Schizandra chinensis showed promise in glucose tolerance tests with rabbits. They suggested that the presence of active principles in these plant extracts might be of use in the treatment of diabetes in man.

The literature on plants claiming antidiabetic properties is voluminous (66) and it is necessary to evaluate those which have been widely reported of value. Although many of the compounds, which were isolated from plant sources, show promise as hypoglycemic agents, further studies could prove them to be undesirable due to side effects or toxicity. However, the compounds could serve as models in the synthesis of active and safe hypoglycemic agents, as they represent new structures (50).

Treatment of diabetes with oral hypoglycemics can be termed only partially successful at the present time. More research is required to produce new compounds which bear more similarity to insulin in their biological action and which are effective in treating more diabetic patients (2).

From recent reviews it is apparent that extensive search for orally active hypoglycemic compounds is being carried out. Consequently testing many chemicals and plant extracts has continued because of the still remaining need for orally active agents with broader spectrum of action than the presently available oral agents (67).

3. Oplopanax horridum (Devil's Club)

Large and Brocklesby (1), in 1938, reported that extracts of Devil's Club root bark exhibited marked hypoglycemic activity, with no apparent toxic effects, when orally administered to normal starved rabbits. Their attention was brought to this material through a surgical patient who developed symptoms of diabetes on hospitalization, and who kept in apparent good health for several years by oral doses of an infusion of this root bark. Such a preparation was used by Pacific Coast Indians, but the specific purpose behind its use was not clear. The active hypoglycemic principle was not isolated in the pure form.

Piccoli, Spinapolice and Hecht (68) reported, in 1940, in a pharmacologic study of Devil's Club, that it had no hypoglycemic effect on normal starved rabbits.

Stuhr and Henry (69), in 1944, worked on three different lots of Devil's Club (British Columbia, Oregon, and commercial). They thought that the previous contradictory findings might have been due to variation in the activity of root bark from different sources. They noted that no apparent variations were observed in the different lots. They also reported that no apparent hypoglycemic effects were observed in normal starved rabbits following oral administration of extracts.

Regarding these contradictory reports about Devil's Club, it was decided to re-evaluate its hypoglycemic activity, using different aqueous extracts, and to compare the activity to tolbutamide.

Devil's Club is a common name for the plant Echinopanax horridum (J.E. Smith) Decne. and Planch. (Panax horridum J.E. Smith, Oplopanax horridum Miq., Fatsia horrida B. and H.) (70). In Japan the plant apparently is called Oplopanax japonicus (Nakai), Oplopanax horridus var. japonicus (Nakai), Echinopanax japonicus (Nakai) (71). This plant belongs to the Araliaceae Family (70, 71), and is called "Suxt" by the Tlingit (72) and "Haribuki" by the Japanese (73).

Devil's Club grows in rocky places, Isles Royale, Lake Superior, Montana to Oregon and Alaska, and Japan.

Family Araliaceae, Ginseng Fam., (Syn. Hederaceae) is a large family including 62 genera and about 750 species, but only four genera occur in the United States

and Canada. Other genera grow in the tropics, specially in India, Malaya, and tropical America (74).

Devil's Club has a very spiny stem and large leaves. It is a shrub about one meter tall with simple stout stem about 1 - 1.5 cm thick, yellowish brown in color. It bears long prickles 5 - 10 mm. The leaves palmately lobed, 7 - 9 lobes (71). Flowers are small greenish white, in small umbels arranged in a panicle, fruit bright red, juicy but not edible. The plant has a strong odor (75).

Devil's Club has been used since ancient times for generations among the Indians in Southeast Alaska (72). The thorny stalks of the plant were used for whipping those suspected of practicing witchcraft (76), and some sick people to get the devil out of their bodies (77). The hunters bathed their bodies in an extract before a hunting expedition for whale or seal (72). The dried bark when mixed with red paint was used as a love charm (78).

Among the medicinal uses of Devil's Club was its application as baby talc and body perfume. Internally it was used to regulate puerperal menstruation, relieve menstrual cramps, and as lactation suppressent (79). The Tlingit and Haida people in Southeast Alaska drink the infusion prepared from the bark or the roots for many purposes such as: cold, arthritis, constipation, general tonic, stomach ulcers, gall stones, and tuberculosis (72).

The tincture prepared from the roots (Tinctura echinopanacis) is used in asthenic conditions and hypotony (80).

Devil's Club is enjoying a revival, currently it is believed to be effective against cancer by some Indians (72).

In Japan the plant is used as antipyretic or cough cure (73).

4. Phytochemical Investigation on Family Araliaceae

Stuhr and Henry (69) reported in a chemical investigation on Devil's Club root bark, from British Columbia, the presence of: glycosidal principles of a saponin-like nature, which were nontoxic to frogs and rabbits, carbohydrates, glycerides, oleic acid and tannins. Alkaloids were not detected by the methods of ether-soluble alkaloids or Stas-Otto. No gallic acid or highly unsaturated fatty acids were present. Extraction with various solvents gave the following results:

Alcohol-soluble extractives	43.91%
Hydro-alcohol soluble extractives	23.43%
Purified benzin-soluble extractives	13.19%
Non-volatile ether-soluble extractives	23.06%
Volatile ether-soluble extractives	4.97%
Water-soluble extractives	9.26%

In Japan Kariyone and Morotomi (81) isolated echinopanacen, a sesquiterpene, $C_{15}H_{24}$, and echinopanacol, a sesquiterpene alcohol, $C_{15}H_{25}OH$, from the steam distilled essential oil of the whole plant. The structures of these compounds were not established. Takeda, Minato and Ishikawa (73) isolated oplopanone, a new sesquiterpene ketone, $C_{15}H_{26}O_2$, from the

essential oil prepared by ether extraction of the roots of the same plant of Kariyone and Morotomi, and which they called Oplopanax japonicus.

A preliminary chemical study of Araliaceae family which included: Echinopanax elatum, Acanthopanax sessiliflorum, Kalopanax recinifolia, Aralia mandsharica and Aralia continentalis, indicated the presence of glycosides and saponins in all species with the exception of A. continentalis which contained only glycosides. The glycosides were of the cardiac stimulant type. All plants were found to contain ethereal oil varying in quantities (82).

In another pharmacognostic study on family Araliaceae which included: Kalopanax septunlobus, Echinopanax elatus, and Aralia continentalis, glycosides predominated. Glycoside derivatives of coumarins, flavonoglycosides, saponins, essential oils, resins, tannins, starch, with traces of alkaloids were also detected (83).

Different investigations on Panax ginseng fam. Araliaceae revealed the presence of choline in the roots 0.1 - 0.2 % w/w (84), sucrose, fructose, glucose, fumaric, succinic, maleic, malic, citric, tartaric, oleic, linoleic, and several other fatty acids, in addition to an essential oil, a yellow pigment and several glucosides of unknown structure (85). A preliminary report on the contents of Panax ginseng root indicated the presence of two unknown sapogenins, which were believed to be of the triterpene type (86). A study of the saponins and sapogenins of ginseng has suggested a

structure for panaxatriol which was obtained by the acid hydrolysis of ginsenoside R (87).

A study on Mackinlaya species resulted in the isolation of two alkaloids which belong to a new class, from the leaves of M. subulata and M. macrosciada (88).

The ether extract of the roots of Panax ginseng was reported to contain sitosterol, β-sitosterol glucoside, together with an essential oil. A sesquiterpene of the formula $C_{15}H_{24}$, Panacen, probably β-elemene (89), was detected in the oil (90).

Compounds of C_{15} were also detected in the volatile oil of Aralia nudicaulis rhizome; a sesquiterpene of the formula $C_{15}H_{24}$, and a C_{15} alcohol presumably $C_{15}H_{25}OH$ (91, 92).

Murray and Stanley (93) reported that the essential oil of Nathopanax simplex consisted largely of myrcene, with smaller amounts of α- and β-pinene, dipentene, aromadendrene, α- and γ-camphorene, paraffin wax and other unidentified terpenes and sesquiterpenes. Two impure bicyclic sesquiterpenes, which were not identified, gave azulene and a little cadalene on dehydrogenation.

PART I

BIOLOGICAL EVALUATION OF EXTRACTS OF

OPLOPANAX HORRIDUM

ROOT BARK

III. DISCUSSION

The hypoglycemic activity of Devil's Club root bark was re-evaluated biologically using normal white rabbits. The experimental design was similar to that adopted in medical research laboratories of Schering A.G. (24), with modifications.

The preliminary screening of different extracts was performed on rabbits which have been starved for 24 hours. Usually a group of six rabbits was used in each biological assay. Two of these were used as controls. They were treated in the same manner but did not receive any extract. This was done to avoid large day-to-day variation (94) in fasting blood glucose values. The other four were given the specified dose of extract by means of stomach tube. Blood samples were taken immediately after oral administration of extracts (95), then one, two, four and six hours following the administration. Blood was taken from the marginal ear vein in duplicate. The mean values are given.

Blood sugar was quantitatively determined either by Folin and Malmros (96) micromethod or by the method of Mendel, Kemp and Myers (97), and expressed as milligrams of glucose per hundred milliliters of blood (mg/100 ml).

The hypoglycemic activity was expressed as the blood sugar lowering effect. The maximal lowering in blood sugar from the initial starvation level was observed. This maximal lowering in rabbits receiving extracts was compared with that of controls.

A standard oral hypoglycemic agent tolbutamide (98) was used in a group of animals. Results are given in Table 1. The maximal lowering in blood sugar of control rabbit was 15 mg/100 ml. The maximal lowering in the blood sugar of rabbit 2 and 3 which received tolbutamide was 51 and 45 mg/100 ml respectively. There was a steady and continuous decrease in blood sugar level during the six hours of observation. It was noticed that in spite of individual variation in response to tolbutamide both rabbits exhibited marked hypoglycemic effect compared to the control. These observations were used as guide lines for detecting hypoglycemic effect in different extracts.

Glucose values within those of controls or less than 10 mg/100 ml were considered insignificant.

As the number of animals used was limited, no statistical evaluations were made.

Table 1

Effects of Tolbutamide on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Kg.	Dose mg/Kg Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1 hr.	2 hr.	4 hr.	6 hr.
1 (control)	3.15	-	85	76	90	70	85
2	3.53	125	92	56	51	50	41
3	3.12	125	105	81	71	60	60

Large and Brocklesby (1) reported that their aqueous extract of Devil's Club produced a rapid reduction in blood sugar of normal starved rabbits. When doses of 0.1, 0.2 or 0.25 ml per pound of body weight were used the lowering in blood sugar ranged from 35-70 mg/100 ml. Doses of 0.4 - 0.5 ml caused initial rise followed by very rapid drop in blood sugar. When doses of 0.8 - 1 ml were given the erratic behaviour was more pronounced but there was lengthened hypoglycemic effect. Large and Brocklesby noted that although there was considerable variation in individual response, all the animals reacted in the same general way towards the extract.

The aqueous extract of Devil's Club, prepared by the procedure used by Large and Brocklesby, was screened for hypoglycemic effects. Results are given in Table 2.

It was observed, excluding rabbit 4 and 6 because of bleeding, that the control rabbit 1 showed a steady sugar level during the six hours. Rabbit 2 showed a maximal lowering of 2 mg/100 ml, while rabbit 5 showed no lowering. Rabbit 3 exhibited a maximal lowering of 29 mg/100 ml from the initial starvation level, which was noticed to be higher than the rest of the group. A lowering of 25 mg/100 ml was observed during the first hour. The blood sugar level remained steady and close to normal values compared with control for the next five hours. This suggested that the high initial blood sugar level might be due to excitement during handling (99). It was concluded that this extract might

Table 2

Effects of Devil's Club Extract Prepared by Large and Brocklesby's Method on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1 hr.	2 hr.	4 hr.	6 hr.
1 (control)	7.5	-	79	92	82	89	85
4 (control)	6.6*	-	84	81	119	105	147
2	7.7	0.25	80	78	83	79	89
3	7.75	0.25	112	88	86	88	83
5	6.9	0.25	84	89	90	84	84
6	6.9*	0.25	83	119	-	99	100

*These two rabbits were bleeding.

have a slight hypoglycemic effect which was demonstrated by rabbit 3. If this effect was compared with that of tolbutamide, it would be described as negligible.

Large and Brocklesby (1) stated in their investigation that the extract used in their experiments was not pure and contained, beside the active material, some gums, sugars, magnesium acetate, small amounts of magnesium hydroxide and other "natural contaminents". In order to minimize the

quantities of substances which might be interfering with the active principle, it was decided to test a refined aqueous extract from which some of the "natural contaminents" were removed. The root bark was defatted by petroleum ether, then treated by ethanol to remove gums and resins before preparing the aqueous extract. Tannins were precipitated by magnesium oxide. Results of using the refined aqueous extract are given in Table 3.

The two control rabbits showed no significant lowering in blood sugar within the period of six hours. The four rabbits received the same dose of extract per pound, but rabbits 2 and 3 were given the extract from which tannins were precipitated. Rabbits 2 and 5 experienced a slow but steady lowering of blood sugar values, which were 10 and 15 mg/100 ml, at the end of six hours. On the other hand rabbits 3 and 6 showed no significant lowering in their blood sugar level. Precipitation of tannins had no effect on blood sugar lowering of extracts.

According to Large and Brocklesby (1) a dose of 0.8 - 1 ml per pound caused initial rise in blood sugar which was followed by lengthened and more profound hypoglycemic effect. So it was decided to try a larger dose of the refined aqueous extract and observe any intensified hypoglycemic response. Results are given in Table 4.

All rabbits of this group including controls exhibited elevation in blood sugar during the six hours. Rabbits 2 and 3, which received the extract from which tannins were

Table 3

Effects of Refined Extract of Devil's Club

on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml								
			Initial	1 hr.	1.5 hr.	2 hr.	2.5 hr.	4 hr.	4.5 hr.	6 hr.	6.5 hr.
1 (control)	8.0	-	86	92		92		91		95	
4 (control)	7.0	-	83	84		80		91		96	
2	8.0	0.3*	92		88		87		86		82
3	8.25	0.3*	86		91		90		86		90
5	7.0	0.3	100	96		98		88		85	
6	7.4	0.3	91	84		89		99		97	

*These extracts were tannin-free

Table 4

Effects of Refined Extract of Devil's Club Using Large Doses on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1 hr.	2 hr.	4 hr.	6 hr.
1 (control)	4.25	-	106	114	104	119	115
4 (control)	4.4	-	97	119	105	108	129
2	4.06	3.0*	90	96	129	122	119
3	4.5	3.0*	81	85	111	100	102
5	4.06	3.0	103	123	128	97	107
6	4.56	3.0	89	125	101	86	116

*These extracts were tannin-free.

precipitated, showed no lowering in blood sugar. Rabbits 5 and 6, which were given the extract without precipitation of its tannins, exhibited a maximal lowering of 6 and 3 mg/100 ml respectively. This insignificant lowering was reached in four hours after which there was a rise again to a level higher than that of the initial starvation. The rise in blood sugar level of rabbits receiving the extracts was within normal values compared with controls.

It was noticed from results of previous biological evaluations, using different aqueous extracts and changing doses, that these extracts had no significant hypoglycemic effect on the blood sugar of normal starved rabbits.

Large and Brocklesby (1) had reported that the aqueous extract of Devil's Club root bark, when fed with glucose to starved rabbits, caused profound lowering in the maximum blood glucose rise, and blood sugar fell rapidly below the initial starvation level within one and a half hours. So the refined aqueous extract was re-evaluated using rabbits with induced alimentary hyperglycemia. Results are given in Table 5.

It was noticed that the blood glucose concentration of the whole group including controls did not fall to the initial starvation level within the period of four hours. The maximum rise in blood sugar was reached after one hour in rabbits 1 (control), 2 and 3, and after two hours in rabbits 4 (control) and 5, and after half an hour in rabbit 6. The oral glucose tolerance test has been criticized because of its excessive variability, generally attributed to inconsistent gastrointestinal absorption (94).

Control rabbits 1 and 4, which received glucose alone, showed maximum glucose rise of 87 and 124 mg/100 ml. Rabbits 2 and 5, which received glucose and the extract in a dose of 0.5 ml per pound, showed a maximum blood glucose rise of 102 and 134 mg/100 ml. Rabbits 3 and 6, which received glucose and the extract in a dose of 1 ml per pound,

showed a maximum blood glucose rise of 123 and 87 mg/100 ml. Comparing the maximum blood glucose rise in each rabbit receiving the extract with those of controls, it was obvious that there was no apparent lowering in maxima.

In conclusion, no improvement in tolerance to orally administered glucose, which can be taken as indication of hypoglycemic activity, was noticed.

Table 5

Effects of Refined Extract of Devil's Club on Blood Sugar in Rabbits with Induced Alimentary Hyperglycemia

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1/2 hr.	1 hr.	2 hr.	4 hr.
1 (control)	6.4	-	113	189	200	189	142
4 (control)	5.8	-	89	154	189	213	143
2	6.1	0.5*	105	197	207	156	147
5	5.8	0.5	103	145	144	237	138
3	6.5	1.0*	119	167	242	193	143
6	5.8	1.0	114	201	178	192	174

*These extracts were tannin-free.

For completeness of screening all the fractions of Devil's Club root bark, it was decided to evaluate the aqueous extract prepared from the alcohol soluble fraction. Results are given in Table 6. In addition the fraction soluble in light petroleum ether was suspended in water and evaluated. The results are given in Table 7.

In Table 6, it was observed that the maximal lowering in blood sugar of control rabbits was 2 mg/100 ml. Rabbit 3 showed a maximal lowering of 8 mg/100 ml, while rabbit 5 exhibited no lowering during the six hours. There was no significant lowering in blood glucose concentration. It was concluded that this extract had no hypoglycemic effect.

Table 6

Effects of Alcohol Soluble Extractives of Devil's Club on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1 hr.	2 hr.	4 hr.	6 hr.
1 (control)	4.87	-	101	106	98	103	102
4 (control)	8.2	-	88	85	87	104	90
3	3.87	0.3	93	93	85	88	90
5	5.37	0.3	87	94	93	94	99

Table 7

Effects of Light Petroleum Ether Soluble Extractives of Devil's Club on Blood Sugar in Normal Rabbits

Rabbit No.	Wt.,Lb.	Dose ml/lb Body Wt.	Mean of Blood Glucose Concentration, mg/100 ml Initial	1 hr.	2 hr.	4 hr.	6 hr.
1 (control)	4.75	-	97	102	121	104	130
4 (control)	4.6	-	110	97	104	102	123
2	3.75	0.6	70	102	117	89	110
5	3.5	0.6	101	109	132	122	135
3	4.75	1.5	98	108	137	107	119
6	4.6	1.5	101	94	117	109	123

From Table 7, it was observed that there was no lowering but a general rise in the blood sugar of almost all the group including the controls. Only one of the controls showed a maximal lowering of 13 mg/100 ml.

When blood sugar level of rabbits receiving the suspension was compared with those of controls, it was obvious that this suspension exhibited no hypoglycemic effect.

IV. EXPERIMENTAL

1. Animals

Normal white rabbits ranging in weight from 1.6 - 3.5 kilograms were used. Prior to biological tests, rabbits were examined physically for injuries or abscesses by palpating (100). A group of six healthy animals, which resisted handling, and had approximately the same weight and age, were chosen randomly for each biological assay.

The food was withheld for 24 hours prior to introduction of extracts. Water was left ad libitum. The animals were weighed, numbered, and their ears shaved. Rabbits numbered one and four were used as controls.

2. Instruments, Equipment and Materials

The following instruments, equipment and materials were used:

Instruments:

Beckman DU Ultraviolet Spectrophotometer

Materials:

Devil's Club root bark (Oplopanax horridum) was purchased from Dominion Herb Distributers, Montreal.

Tolbutamide U.S.P. was kindly supplied by Hoechst Pharmaceuticals, Montreal.

Heparin solution was obtained commercially (Upjohn Company of Canada).

Equipment:

Soxhlet apparatus (capacity 100 gm)

A canvas cat-restraint bag (Atchison Leather, Atchison, Kansas)

A wooden speculum about 6 x 3/4 x 1/4 inch with a suitable size opening

A stomach tube (Levin duodenal tube), No. 12 French rubber

Corning glass disposable micropipettes, 0.1 ml capacity

3. Extracts

Extracts and suspensions were prepared as near to the time of administration as possible, and refrigerated to avoid possible deterioration in the active principle (101). Aqueous extracts were, in most cases, prepared the day before administration.

Tolbutamide suspension:

Tolbutamide suspension was prepared by suspending tolbutamide powder in water containing 40% polyethylene glycol 400 (101). Each ml of the suspension was equivalent to 125 mg of tolbutamide. The dose given was 125 mg per Kg of body weight. Blood sugar values were determined by Folin and Malmros micromethod (96).

Aqueous extract prepared by Large and Brocklesby's method:

The dried root bark was boiled three times with twice

its volume of water and filtered. Tannins were precipitated by neutral lead acetate, the excess of which was removed by hydrogen sulfide. The excess hydrogen sulfide was removed by boiling. The extract was concentrated by evaporation to a volume in milliliters equal to the original weight in grams. The extract was rendered slightly alkaline by adding magnesium oxide, then filtering. A dose of 0.25 ml per pound was administered. Blood sugar values were determined by Folin and Malmros micromethod.

Refined aqueous extract:

This extract was prepared in a way to minimize "natural contaminents", and added chemicals which might interfere with the active principle. The coarsely powdered root bark was treated with light petroleum ether to remove ethereal and fatty oils, and wax (102). Then it was treated with ethanol to remove substances such as resins, tannins, bitter principles, and alkaloids (103). The marc left was used to prepare the aqueous extract by the method used by Alaskan Indians (72).

Two Soxhlet apparatus were packed, each with 100 gm of coarsely powdered root bark. A volume of approximately 1300 ml of light petroleum ether, boiling point 37-50°C, was used in each apparatus. Extraction was continued for 48 hours, until complete exhaustion was effected, using fresh solvent every 24 hours. Extraction with about 1300 ml of 95% ethanol was continued, after drying the marc in a fumehood. Fresh solvent was used every 24 hours for 5 days.

Complete exhaustion, by solvent, was tested by evaporating a small portion of the menstruum on a watch glass. The combined marc was dried at room temperature, and transferred to a 5 liter round bottomed flask. Three liters of water were added and the mixture heated. Temperature was maintained at 80-90°C for 4 hours. The extract was strained, and concentrated under reduced pressure. The volume of the concentrated extract was reduced to 200 ml by evaporating on a water bath. One ml of this extract was equivalent to 1 gm of the dry root bark. A dose of 0.3 ml per pound was given, and blood sugar values were determined by Folin and Malmros micromethod. Doses of 3 ml per pound, and 0.5 and 1 ml per pound with 0.5 gm of glucose per pound were administered. Blood sugar values were determined by Mendel, Kemp and Myers' method (97).

Precipitation of Tannins:

Heavy magnesium oxide (104) was used for precipitating tannins. This method was cleaner, faster and easier than the hydrogen sulfide method. For each gm of the dry extract, 300 mg of heavy magnesium oxide was used. The calculated amount was added to the aqueous extract and warmed, with stirring. The precipitated tannins and excess magnesium oxide were removed by filtration.

Aqueous extract of alcohol soluble extractives:

A weight of 200 gm of the coarsely powdered root bark was treated as under refined aqueous extract. The combined alcoholic extract was concentrated under reduced pressure.

The brown residue left was extracted with water and filtered to yield an aqueous extract in which 1 ml was equivalent to 1 gm of the root bark. A dose of 0.3 ml per pound was administered. Blood sugar values were determined by Folin and Malmros micromethod.

Aqueous suspension of light petroleum ether soluble extractives:

Two hundred grams of coarsely powdered root bark were treated as under refined aqueous extract. The combined light petroleum ether extract was freed from solvent under reduced pressure. The residue left was suspended in water containing 40% polyethylene glycol 400 to a volume of 200 ml. Each ml of the suspension was equivalent to 1 gm of the root bark. Doses of 0.6 and 1.5 ml per pound were administered. Blood sugar values were determined by Folin and Malmros micromethod.

4. Route of Administration and Dosage

Fluids were administered orally by means of stomach tube. Rabbits were securely held in a canvas bag. A wooden speculum with a suitable sized opening was placed in the rabbit's mouth behind the incisors. A number 12 French rubber tube was threaded through the opening in the speculum. As the tube passed over the base of the tongue it was swallowed slowly. When the tube was securely in the rabbit's stomach, the fluid was introduced by means of a syringe inserted into the free end of the tube.

5. Blood Samples

Blood samples were taken from the marginal ear vein. The shaved lateral margin of the ear was cleaned with 70% ethanol. Xylene was swabbed on the ear vein to increase the flow of blood. A sterile needle was used to puncture the vein (100). It was noticed that incision caused bleeding in some rabbits because of large longitudinal cuts. Therefore the venipuncture method was followed in most cases. Micropipettes were dipped in heparin solution before withdrawing duplicate 0.1 ml blood samples. Blood samples were placed in numbered centrifuge tubes containing deproteinizing solution, covered with parafilm, and shaken immediately. Samples were centrifuged at 3000 r.p.m. for 5 minutes. The supernatant solutions were transferred into dry clean previously numbered test tubes for analysis of glucose content.

6. Blood Sugar Analysis

Two methods were used.

Folin and Malmros (96) micromethod was first followed. Blood sugar was oxidized with alkaline potassium ferricyanide. The ferrocyanide produced was converted to Prussian blue and measured photometrically.

Reagents:

Sodium tungstate 10% solution

Sulfuric acid 2/3 N

Dilute tungstic acid solution was prepared from the

above solutions by adding 20 ml of the first solution to a liter volumetric flask, diluting to about 800 ml and adding, with shaking, 20 ml of 2/3 N sulfuric acid, and completing to volume.

Potassium Ferricyanide solution

Sodium cyanide carbonate solution

Ferric iron solution

Procedure

Dilute tungstic acid was used as deproteinizing solution. Exactly 0.1 ml blood sample was added to the centrifuge tube containing 9.9 ml dilute tungstic acid solution and mixed immediately. After centrifugation a volume of 4 ml of the clear supernatant fluid was transferred to Folin test tube containing 2 ml 0.4% potassium ferricyanide and 1 ml cyanide carbonate solution. Folin tube was heated immediately in a boiling water bath for 8 minutes, then cooled in running water for 2 minutes. A volume of 5 ml of ferric iron solution was added, mixed, and let stand for two minutes, then diluted to nearly 25 ml, two drops of ethanol added to cut the foam, and finally was diluted to the mark. The solution was allowed to stand for 10 minutes, then its optical density was measured at 520 mμ. The spectrophotometer was set to zero with water.

Standard glucose solution:

An accurate weight of 100 mg anhydrous glucose was dissolved in distilled water, and made up to 100 ml, using volumetric flask.

Calibration:

Volumes of 0.05, 0.08, 0.1, 0.12, 0.15, 0.18, 0.2, 0.22, 0.25, 0.28, 0.3 and 0.33 ml of the standard glucose solution were diluted to 10 ml each with distilled water. Four ml of the resulting solution was introduced to a Folin test tube containing 2 ml 0.4% potassium ferricyanide and 1 ml cyanide carbonate solution, heated in a boiling water bath, and treated as above.

A standard curve was constructed, mg of glucose vs. absorbance, to confirm the validity of Beers law in this range of concentration. It was noticed that best results were obtained in the range 0.06 - 0.18 mg of glucose.

Reagents for this method were numerous and solutions had to be freshly prepared. The procedure was long and time consuming.

Mendel, Kemp and Myers' method (97) was simple. A pink complex was formed as a result of the reaction between sulfuric acid and glucose. The optical density of this colored complex was measured at 520 mμ. It has been reported that in the presence of chlorides the formation of color was inhibited.

Reagents:

Sulfuric acid specific gravity 1.84

Trichloroacetic acid

Silver sulfate

Benzoic acid

Procedure:

Deproteinizing solution was prepared by dissolving 5 gm of trichloroacetic acid in 50 ml distilled water, adding 0.1 gm silver sulfate and dissolving. The solution was made up to 100 ml by distilled water.

An exact volume of 0.1 ml blood sample was added to the centrifuge tube containing 1.9 ml of the deproteinizing solution, mixed, then centrifuged. One ml of the supernatant fluid was transferred to a wide test tube containing 3 ml of sulfuric acid, specific gravity 1.84, mixed immediately, then placed in a vigorously boiling water bath for exactly 6.5 minutes. The test tube was cooled in running water. The optical density of the solution was measured at 520 mμ. The spectrophotometer was set to zero with the same solution containing no blood.

Standard glucose solution:

A saturated solution of benzoic acid was prepared by dissolving 2.5 gm in one liter of boiling distilled water. This solution, after cooling and filtering, was used as a solvent for preparing the standard glucose solution (100 mg of anhydrous glucose per 100 ml).

Calibration:

Volumes of 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 and 0.16 ml of the standard glucose solution were diluted to 2 ml each with distilled water. One ml of the resulting solution was introduced to a test tube containing 3 ml sulfuric acid, specific gravity 1.84, mixed, and heated in boiling water

bath, and treated as before. The spectrophotometer was set to zero with sulfuric acid of the same concentration.

In glucose tolerance test the required range of standard glucose solution was 0.08 - 0.26 mg anhydrous glucose per 2 ml of saturated benzoic acid solution.

Calibration was done just before or after each biological assay. In almost all cases good calibration curves were obtained, from which milligrams of glucose in blood samples were deduced.

All reagents were analytical reagent grade.

V. SUMMARY

Devil's Club root bark was biologically re-evaluated for hypoglycemic activity.

Experimental conditions similar to those used by Large and Brocklesby (1), and modifications of extracts and doses, revealed no hypoglycemic activity.

PART II

COMPOSITION OF THE VOLATILE OIL OF

OPLOPANAX HORRIDUM

ROOT BARK

VI. INTRODUCTION

As a knowledge of chemical constituents of plants is desirable not only for the discovery of new therapeutic agents, but also for the discovery of sources of economic materials, and precursers of complex chemical substances (50), the volatile oil of Devil's Club root bark was analyzed. The quantity of the essential oil, prepared by steam distillation, was not enough for systematic physical and chemical tests. So the combined gas-liquid chromatography with mass spectrometry, which represents a powerful technique for the analysis of complex mixtures (105), together with other analytical instruments were relied upon during this investigation.

Essential oils, or volatile oils are found in various plant organs and tissues. They usually constitute the odorous principles of the plants (106). They are considered to be end products of metabolism (107), which may serve in the fertilization process and protection of the plant from enemies.

Volatile oils are used therapeutically (108), either internally or externally. Some have antimicrobial properties (109).

Volatile oils are generally mixtures of hydrocarbons and their oxygenated derivatives (108). A diverse group of compounds occur in these oils (110): hydrocarbons, alcohols, acids, esters, aldehydes, ketones, phenols and phenol ethers,

oxides, lactones, nitrogen compounds and sulfur compounds. The hydrocarbons of chief importance are the terpenes ($C_{10}H_{16}$) and the sesquiterpenes ($C_{15}H_{24}$) (106). The hydrocarbons $C_{15}H_{24}$ - $C_{15}H_{18}$, related alcohols, ketones and a few other types have been isolated commonly from essential oils. Lactones are common in oxygenated sesquiterpenes (111).

VII. DISCUSSION

The essential oil under investigation was prepared by steam distillation of the dry root bark of Oplopanax horridum. The average yield was 1.37% w/w of yellow colored oil. The steam distilled oil was separated into two portions by treating with sodium carbonate solution. The non-acid portion which represented 60% w/w of the oil was investigated.

The gas-liquid chromatography technique was used to separate and collect components of the essential oil. An F and M model 500 gas chromatograph, equipped with thermal conductivity detector, was employed, using helium as carrier gas. Variations in: column composition, column temperature, attenuation, and volume of injected sample were made until a satisfactory resolution was obtained. Thirty three measurable peaks, on three different columns (C-20M, SE-30, and Lac-728) were observed. The minute peaks which could not practically be measured were neglected. The best resolution of peaks was obtained on Lac-728 column. The results are given in the order in which the various constituents were eluted from that column in Figure 1. The retention times, peak areas and area percentage are shown in Table 8 and 9 respectively.

For a preliminary identification of the components of the oil, different known compounds were injected with the oil and the augmented peaks were observed. Results

of peak augmentation on Lac-728 are given in Table 10.

For identification purposes the fractions emerging from the Lac-728 column were collected, when possible, and subjected to infrared, ultraviolet, and high-resolution mass spectrometric analyses. When there was enough sample a nuclear magnetic resonance spectrum was recorded. The mass spectra of almost all the fractions were also recorded on A.E.I. MS12 mass spectrometer coupled to Aerograph model 1200 Hy-Fi III gas chromatograph (figure 1a).

The molecular ions of the components, in most cases, were assigned to the highest mass ions in the spectra (112, 113), excluding heavy isotopes, from which the observed fragment ions immediately below could logically be formed (114, 115).

The molecular formulas of the components were determined by one or more of the following methods:
accurate mass measurements by the high-resolution mass spectrometer,
calculation when the isotope peak intensities were large enough (116),
deducing the elemental composition of prominent fragment ions, either from their isotope peak intensities (117) or from reference appendix of common fragment ions (118), and reconstruction of these fragments,
the fragmentation pattern (119), and reference appendices of common fragment ions (118), and common fragments lost (120).

The aliphatic hydrocarbon moiety of the components was indicated by the presence of clusters of peaks 14 mass units apart (121, 122, 123), and each having the species C_nH_{2n+1} or C_nH_{2n-1} as the most abundant ion. The most intense fragment ions were at C_3 and C_4. By tracing the $(C_nH_{2n+1})^+$ homologous series of ions beginning from CH_3+, the number of carbon atoms could be deduced up to $C_7H_{15}+$ (124). The number of carbon atoms could also be deduced by tracing the tropylium ion C_7H_7+ (m/e 91) or substituted tropylium ion $(91+14n)^+$.

The presence of oxygen was deduced from prominent peaks at m/e 31, 45, 59, 73 etc. If m/e 31 was larger than 2.2% of m/e 30 and over 0.012% of the height of m/e 29 this was taken as conclusive evidence of the presence of oxygen (125, 126, 129). Examination of the peak at m/e 43 indicated the presence of oxygen in some cases. If the fragment ion m/e 44 was 2% of that of mass 43 this was proof that mass 43 was almost entirely due to C_2H_3O+ and not C_3H_7+ (127, 128). When the high-resolution showed a split peak at m/e 43 the peak at the lower mass was due to C_2H_3O+, while that at the higher mass was due to C_3H_7+ (C_2H_3O = 43.018390, C_3H_7 = 43.054775) (129). The presence of oxygen could also be deduced from the fragmentation pattern and the presence of certain peaks such as 47, 61, 75, 89, 103, 117, etc., which correspond to $(RCOO+2H)^+$ (130).

All the components of the volatile oil of Devil's Club were found to contain only the elements of carbon,

hydrogen, and, in some instances, oxygen.

The presence of nitrogen was excluded because the molecular ions were at even mass numbers (131) and the largest peaks of the spectra at odd mass numbers (132), with the absence of peaks at half-masses at low mass numbers (133).

Sulfur was not detected by any isotopic clusters in the spectra (134, 119).

The degree of unsaturation, as contained in double bonds and/or rings in the molecule, was calculated from the equation (135):

$$D.B.E. = \frac{(2 \times Carbons + 2) - Hydrogens}{2}$$

The results and conclusions for each fraction will be discussed individually in the following pages. The retention times are those observed using Lac-728 column. The mass spectra of oplopanone will be discussed at the end of this chapter.

A summary of the composition of the essential oil is shown in Tables 11 and 12. Table 11 contains components of lower molecular weights and in the order of their elution from the Lac-728 column. Table 12 contains components of higher molecular weights grouped according to their molecular weights or other common mass spectral features.

Fractions 28 and 32 were not subjected to any instrumental analysis other than gas-liquid chromatography.

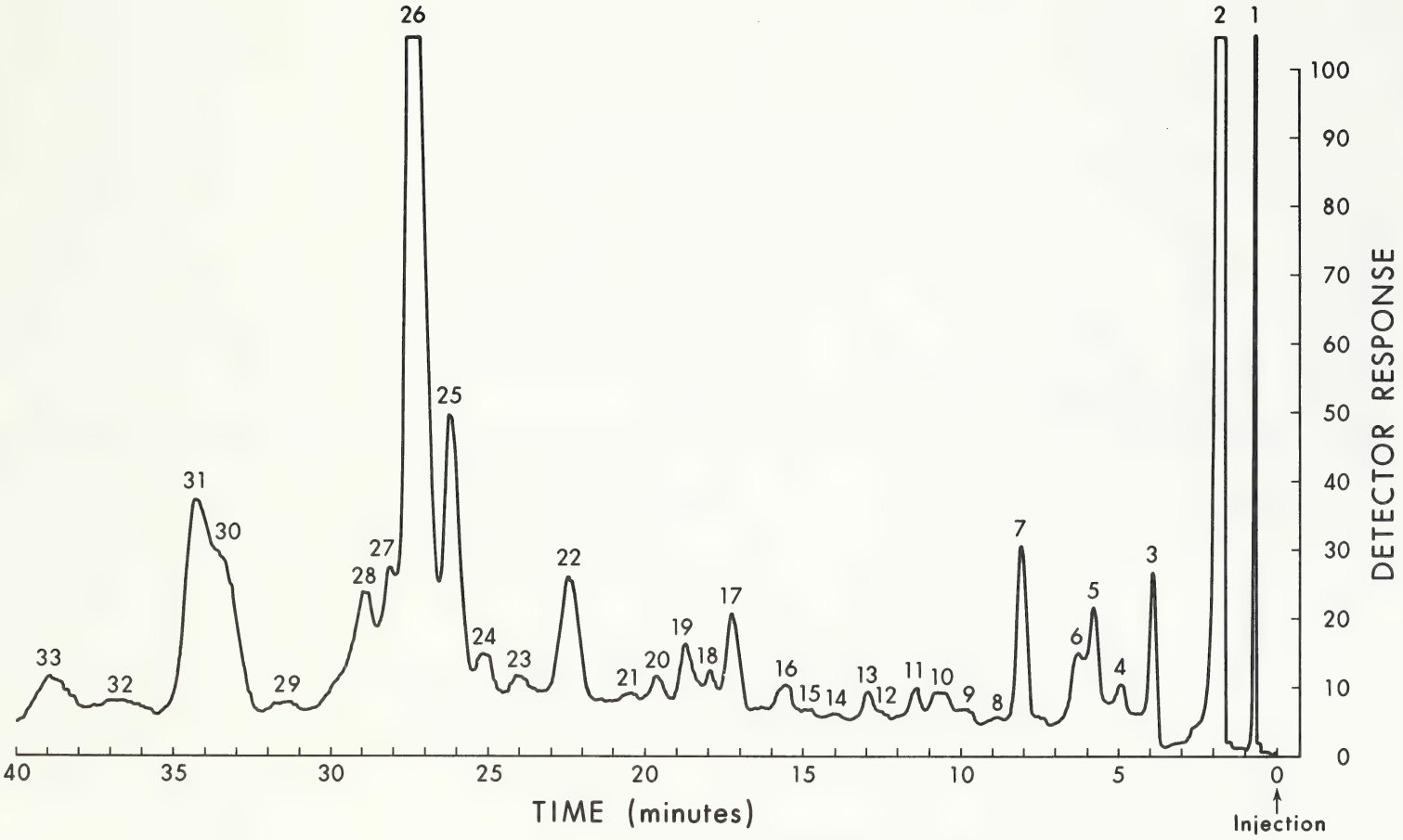


Figure 1. Gas Chromatogram of Oil of Devil's Club Root Bark Using Lac-728 Column (F and M 500 Gas Chromatograph)

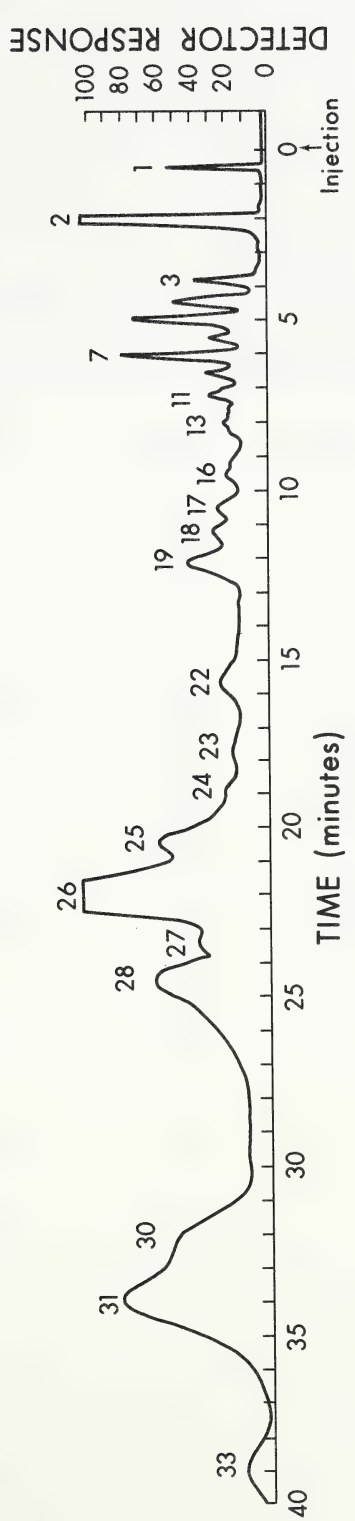


Figure 1a. Gas Chromatogram of Oil of Devil's Club Root Bark Using Lac-728 Column (Aerograph 1200 Hy-Fi III Gas Chromatograph)

Table 8

Retention Times (in mins.) of Constituents of Devil's Club Oil Chromatographed on Lac-728 Column

Peak	Time	Peak	Time	Peak	Time
1	0.6	12	12.5	23	24.4
2	1.7	13	13.0	24	25.5
3	3.7	14	14.1	25	26.6
4	4.7	15	14.9	26	27.8
5	5.6	16	15.7	27	28.4
6	6.1	17	17.4	28	29.3
7	8.0	18	18.1	29	31.9
8	8.8	19	19.0	30	33.9
9	9.7	20	19.9	31	34.6
10	10.5	21	20.7	32	36.8
11	11.4	22	22.8	33	39.0

Table 9

Peak Areas (in mm^2) and Percentages of Constituents of Devil's Club Oil Chromatographed on Lac-728 Column

Peak	Peak area	(%) in Sample	Peak	Peak area	(%) in Sample
1	267	1.24	18	252	1.17
2	5396	24.99	19	456	2.11
3	201	0.93	20	336	1.56
4	161	0.75	21	441	2.04
5	270	1.74	22	682	3.16
6	210	0.97	23	392	1.82
7	355	1.64	24	420	1.95
8	120	0.56	25	1008	4.67
9	135	0.63	26	2556	11.84
10	252	1.17	27	924	4.28
11	168	0.78	28	767	3.55
12	112	0.52	29	468	2.17
13	168	0.78	30	2652	12.28
14	224	1.04	31		
15	176	0.82	32	684	3.17
16	308	1.43	33	638	2.96
17	392	1.82			

Table 10

Peak Augmentation Using Lac-728 Column

Injected compound	Peak augmented
Diethyl ether	1
d-Limonene	4
Methyl heptenone	10
Fenchone	12
l-Bornyl acetate	16
Terpinyl acetate	19
Pulegone	
Geraniol	22
Safrole	25
Methyl eugenol	29
Eugenol	33

Table 11

Composition of Oil of Devil's Club Root Bark

(Components of Lower Molecular Weights)

Peak No.	(%)	No. of Components	Molecular Weights	Molecular Formulas	Identity
1	1.24	1	74	$C_4H_{10}O$	Diethyl ether
2	24.99	1	46	C_2H_6O	Ethanol
3	0.93	2 (a)	72	C_4H_8O	n-Butyraldehyde
		(b)	82	C_6H_{10}	Hexadiene
4	0.75	1	156	$C_{10}H_{20}O$	Unsaturated primary alcohol
5	1.74	2	130	$C_7H_{14}O_2$	Probably 1,1-diethoxy-3-propene,
			130	$C_7H_{14}O_2$	ethyl ester of fatty acid
6	0.97	1	156	$C_{10}H_{20}O$	Unsaturated primary alcohol
Pre 7	Trace	2	144	$C_8H_{16}O_2$	Probably 1,1-diethoxy-3-butene,
			144	$C_8H_{16}O_2$	propyl ester of fatty acid
7	1.64	1	128	$C_8H_{16}O$	Octanal

continued

Table 11 - continued

Peak No.	(%)	No. of Components	Molecular Weights	Molecular Formulas	Identity
8	0.56	2	156	$C_9H_{18}O_2$	Probably 1,1-diethoxy-3-methyl-
			156	$C_9H_{18}O_2$	3-butene, butyl ester of fatty acid
9	0.63	More than one	158	$C_9H_{18}O_2$	Probably methyl ester of fatty acid
10	1.17	1	142	$C_9H_{18}O$	2-Nonanone
12	0.52				Unidentified
13	0.78	More than one	130	$C_7H_{14}O_2$	Probably isoamyl acetate
14	1.04		170		Unidentified

Table 12

Composition of Oil of Devil's Club Root Bark

(Components of Higher Molecular Weights)

Peak No.	(%)	No. of Components	Molecular Weights	Molecular Formulas	Identity
11	0.78	Probably more than one	204		Unidentified
15	0.82	Probably more than one	204		Unidentified
16	1.43				Unidentified sesquiterpene
17	1.82	1	204		Unidentified
18	1.17	1	204	$C_{15}H_{24}$	Probably bicyclic sesquiterpene hydrocarbon
19	2.11	Probably more than one	204		Unidentified
20	1.56	1	204	$C_{15}H_{24}$	Sesquiterpene hydrocarbon

continued

Table 12 - continued

Peak No.	(%)	No. of Components	Molecular Weights	Molecular Formulas	Identity
21	2.04	1	204	$C_{15}H_{24}$	Bicyclic sesquiterpene hydrocarbon, probably, of the cadinane type
31	8.0	1	204	$C_{15}H_{24}$	Bicyclic sesquiterpene hydrocarbon, probably, δ-cadinene
Pre 22	Trace				Probably sesquiterpene alcohol
23	1.82	1	238		Probably sesquiterpene alcohol
30	4.28	More than one	220	$C_{15}H_{24}O$	Sesquiterpene alcohol, probably, of the cadinane type
22	3.16	More than one	236	$C_{15}H_{24}O_2$	Sesquiterpene γ-lactone
24	1.95	1	238		Unidentified oxygenated sesquiterpene

continued

Table 12 - continued

Peak No.	(%)	No. of Components	Molecular Weights	Molecular Formulas	Identity
25	4.67	1	238	$C_{15}H_{26}O_2$	Oxygenated sesquiterpene
26	11.84	More than one			Unidentified oxygenated sesquiterpene
27	4.28				Unidentified oxygenated sesquiterpene
28	3.55				Unidentified
29	2.17				Unidentified oxygenated sesquiterpene
32	3.17				Unidentified
33	2.96	More than one			Unidentified oxygenated sesquiterpene

Fraction 1

Results

Gas-liquid chromatography: this fraction had a very sharp symmetric peak, a retention time of 0.6 min., and represented 1.24% of the essential oil. The peak was augmented by diethyl ether. No attempt to collect this fraction was made because of its high volatility and low percentage.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
14	1.4	30	2.5	47	0.2
15	6.2	31	100.0	50	0.2
16	0.4	32	1.4	51	0.3
17	0.83	33	0.2	59	46.2
18	3.9	41	4.6	60	1.5
19	2.3	42	2.3	61	0.5
26	6.2	43	3.8	69	0.2
27	26.2	44	3.1	73	2.5
28	7.7	45	38.5	74	25.4
29	53.8	46	0.8	75	1.2
				76	0.2

M(74) = 25.4

M+1(75) = 1.2

M+2(76) = 0.2

Isotope abundances:

m/e	% of M
74(M)	100.
75(M+1)	4.72
76(M+2)	0.74

Base peak: at m/e 31

Molecular formula: $C_4H_{10}O$

Double bond equivalents: none

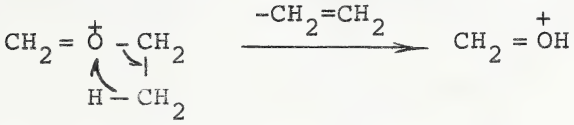
Discussion and conclusions

The molecular ion is most probably m/e 74. The isotopic abundances indicated the formula $C_4H_{10}O$ for this ion. As the component of this fraction is saturated and contains only one oxygen atom it may be either an alcohol or ether (136). This is supplemented by the base peak (m/e 31) and the intense peaks at m/e 45, 59 and 73 (137, 124). The base peak at m/e 31 (CH_3O+) can be derived from primary alcohols $R\!\!\sim\!\!CH_2OH$ or from ethers through rearrangement $R\!\!\sim\!\!CH_2O\!\!\sim\!\!R'$ (138).

The relative stability of the molecular ion (139), the absence of M-18 peak from loss of water (140), and the absence of a prominent peak at M-31, excluded the probability of a primary alcohol.

The three possible isomeric ethers having the formula $C_4H_{10}O$ are: diethyl ether, methyl propyl ether, and methyl isopropyl ether. The last isomer may be confidently eliminated because of the absence of a prominent peak at m/e 43 due to the isopropyl ion, and it does not account logically for the intense peak M-29. To narrow down the possibilities to one, the fragmentation pathway common to ethers (141, 142) was applied to both structures. The diethyl ether structure accounted better for all the fragment ions. Moreover there was a diffuse peak at m/e 16.3 confirming the following transition:

$$59^{+} \longrightarrow 31^{+} + 28$$



m/e 59 m/e 31

By comparing the ten most abundant peaks of this fraction with those of methyl n-propyl ether, methyl isopropyl ether and diethyl ether (143), the conclusion was that Fraction 1 is composed of diethyl ether.

Ethyl ether does not seem to be a common component of essential oils, and its origin is most probably the solvent used for extraction.

Fraction 2

Results

Gas-liquid chromatography: this fraction had a very sharp symmetric peak, a retention time of 1.7 mins., and represented 24.99% of the oil. Because of its great volatility this fraction was collected by a special trap (see Experimental) and no further purification was attempted, but when tested on the Se-30 column it gave a single sharp peak. The collected liquid was colorless, very volatile, with penetrating smell.

Mass spectral data: two different scans were run on MS12 and one on MS9. Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	14	3.6	16	0.5
13	1.2	15	7.9	17	0.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
18	0.8	30	5.7	42	3.9
19	3.6	31	100.0	43	9.6
26	8.6	32	1.2	44	2.5
27	25.0	33	0.2	45	35.7
28	6.8	34	0.3	46	14.3
29	21.4	35	1.6	47	0.6
				48	0.04

An accurate mass determination of the molecular ion (m/e 46) was made by MS9.

Base peak: at m/e 31

Molecular formula: C_2H_6O

Double bond equivalents: none

Ultraviolet data: no UV maximum above 204 mμ.

Infrared data: chloroform solution in potassium bromide microcell of 0.1 mm path.

3620 (m, sharp), 3360 (s, broad), 2880 (s), 2965 (w), 2460 (w), 1620 (w), 1440 (m), 1380 (m), 1310 (w), 1220 (m, broad), 1075 (s), 1035 (v), 865 (s, sharp) cm^{-1}.

Nuclear magnetic resonance data: the following signals were observed:

two-proton quartet at τ6.3-6.4, one-proton singlet at τ7.4-7.5, three-proton triplet at τ8.8. On shaking with deuterated water the one-proton singlet at τ7.4-7.5 was removed to τ5.4.

Discussion and conclusions

The high-resolution mass spectrometry established the

formula C_2H_6O for the molecular ion m/e 46. Again a saturated component containing one oxygen atom and having a base peak at m/e 31 as fraction 1. So this fraction may contain a primary alcohol or an ether. The two possible structures are ethanol and dimethyl ether.

The more plausible structure is ethanol because of the distinct peaks at M-1, M-18 and M-31. It also accounts for all the major fragment ions through the fragmentation pathway common to saturated short chain alcohols (144).

The infrared spectrum showed a broad absorption band at 3360 cm^{-1} (intermolecular hydrogen bonded hydroxyl group), and a sharp band at 3620 cm^{-1} (free OH).

The NMR signals are typical of ethanol.

As all these data were in favor of ethanol, an infrared spectrum of an authentic sample of ethanol was recorded and compared with that of Fraction 2. The two spectra were completely superimposable indicating that this fraction consists almost entirely of ethanol.

To exclude the probability of any contamination with ethanol through solvent ether, during the extraction process, the solvent used was subjected to NMR analysis. The absence of the one-proton singlet from the spectrum was a strong evidence of the absence of ethanol.

Ethanol has been observed in few cases as a normal constituent of essential oils (145). It was reported present in oil of Indigofera, in the fruit of Morinda citrifolia, Eucalyptus amygdalina and Heracleum giganteum.

Ethanol is more commonly found esterified with fatty acids. However the occurance of both free alcohols and free acids may be attributed to hydrolysis of esters during the process of distillation (146, 147). The presence of ethanol may also be due to fermentation of the carbohydrates, present in the root bark, previous to distillation (146).

The exact source of ethanol in the essential oil of Devil's Club root bark is not known.

Fraction 3

Results

Gas-liquid chromatography: this fraction had a sharp symmetric peak, a retention time of 3.7 mins., and represented 0.93% of the essential oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.2	38	3.1	54	4.7
13	0.2	39	38.9	55	19.1
14	1.4	40	7.3	56	77.1
15	7.1	41	92.2	57	54.4
26	6.2	42	17.1	58	8.0
27	61.1	43	73.3	59	0.7
28	9.6	44	100.0	67	8.9
29	60.7	45	19.6	69	2.2
30	2.7	46	0.9	70	1.1
31	4.4	47	0.7	71	7.8
37	1.1	51	2.2	72	15.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
73	0.9	79	0.2	82	12.9
74	0.2	80	0.2	83	0.7
75	0.2	81	1.8		

(a) M(72) = 15.3

M+1(73) = 0.9

M+2(74) = 0.2

Isotope abundances:

m/e	% of M
72(M)	100.
73(M+1)	5.86
74(M+2)	1.3

Base peak: at m/e 44

Molecular formula: C_4H_8O

Double bond equivalents: one

(b) M(82) = 12.9

M+1(83) = 0.7

Isotope abundances:

m/e	% of M
82(M)	100.
83(M+1)	5.43

Base peak: at m/e 41

Molecular formula: C_6H_{10}

Double bond equivalents: two

Discussion and conclusions

A quick inspection of the spectrum of this fraction, starting at the high mass end, indicated the presence of

more than one component. The appearance of peaks at m/e 72 and 82 with 10 mass units difference can not possibly be due to an eliminated fragment (148). The isotopic abundances indicated the elemental composition C_4H_8O (a) and C_6H_{10} (b) for the molecular ions 72 and 82.

Component a

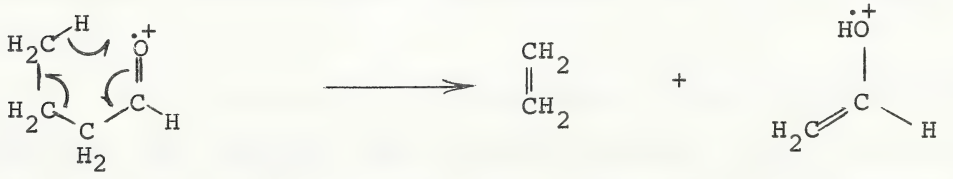
The base peak at m/e 44 most probably belongs to component a rather than b because of the reasonable loss of 28 mass units but not 38. The formula, C_2H_4O, of the base peak could be derived from structures such as (149, 150):

$OCH-CH_2\}R\{H$ (rearrangement)

$CH_2=CHO\}R\{H$ (rearrangement)

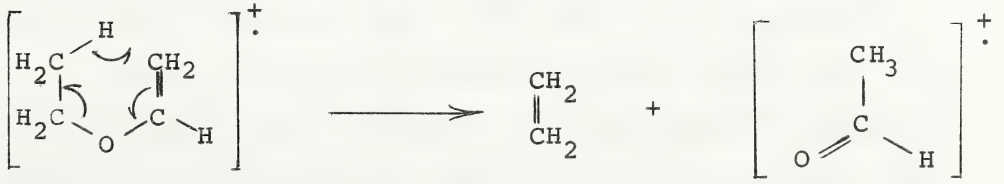
Component a might be an aldehyde or unsaturated ether. The two possible structures capable of undergoing McLafferty rearrangement (151, 152) to give m/e 44 as the base peak are n-butyraldehyde and ethyl vinyl ether.

n-Butyraldehyde



m/e 44

Ethyl vinyl ether



m/e 44

On comparison of the ten most abundant peaks of n-butyraldehyde (153) and ethyl vinyl ether (154) with those of component a it seemed probable that a was n-butyraldehyde. The mass spectrum was also similar to that of n-butyraldehyde (151).

The presence of n-butyraldehyde has been reported in the volatile oils of Eucalyptus globulus (155), Melaleuca leucadendron (156) and Monarda festulosa. It was also reported present in the volatile oil of Lavendula delphinensis (157) and Artemesia scoparia (158).

Component b

The base peak of this component could be at m/e 56 from the loss of CH≡CH, or at m/e 41 from the loss of $C_3H_5\cdot$ from the molecular ion (m/e 82). However it should be taken into consideration that the fragment ion 41 could partly be derived from component a.

Component b having the formula C_6H_{10} may be a diene, an alkyne, cycloalkene, or cycloalkane with olefinic substituent.

On the basis of the intensities of the molecular ion, $[M-CH_3]$, and $[M-C_2H_4]$ ions, all the possible cyclic structures may be safely excluded (159). The hexynes, whose base peaks are at $M-CH_3$ (160), and the conjugated dienes which have stable molecular ions (161), may also be excluded.

Both 1,4 and 1,5-hexadienes could account for the major fragment ions. However it is difficult to conclude that b is one or the other due to the great tendency for

rearrangements in unsaturated hydrocarbons (165).

The base peak of 1,5-hexadiene is at m/e 41 (160). The presence of 1,5-hexadiene (diallyl) has been reported in the volatile oil of Ormensis multicaulis (162).

In conclusion, Fraction 3 contains two components which are most probably n-butyraldehyde and a hexadiene which may be diallyl.

Fractions 4 and 6

As these two fractions had virtually identical mass spectra, they might be treated as isomers (163). The noticeable quantitative differences in the abundance of certain fragments may be an indication of geometrical isomerism (164).

Fraction 4

Results

Gas-liquid chromatography: the peak corresponding to this fraction was relatively small, and had a retention time of 4.7 mins., and represented 0.75% of the oil. The retention data suggested d-limonene.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.7	16	0.4	28	3.9
13	0.7	19	1.1	29	51.1
14	2.5	26	8.6	30	5.0
15	7.9	27	62.5	31	26.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
32	0.4	65	10.4	95	31.8
38	4.6	66	6.7	96	2.9
39	48.2	67	100.0	97	2.5
40	9.6	68	15.0	98	0.4
41	66.1	69	8.9	99	2.1
42	9.3	70	2.5	101	4.6
43	50.0	71	16.4	109	4.3
44	7.5	73	1.1	110	14.6
45	11.4	74	0.4	111	21.1
46	3.6	77	11.1	112	3.2
47	3.2	78	2.5	113	7.8
49	0.7	79	20.7	114	0.7
50	5.4	80	4.6	115	0.4
51	11.4	81	53.3	128	1.8
52	5.7	82	31.4	129	8.2
53	38.6	83	17.1	130	0.7
54	13.2	84	2.1	131	0.5
55	55.7	85	11.4	138	0.7
56	6.1	86	16.8	141	32.1
57	30.4	87	1.1	142	3.2
58	38.9	88	0.4	143	0.5
59	4.3	91	7.1	155	0.7
60	0.4	93	24.6	156	0.4

M(156) = 0.4

Base peak: at m/e 67

Molecular formula: $C_{10}H_{20}O$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
59.7	$110^+ \longrightarrow 81^+ + 29$
54.7	$82^+ \longrightarrow 67^+ + 15$
25.1	$67^+ \longrightarrow 41^+ + 26$
36.4	$83^+ \longrightarrow 55^+ + 28$
89.4	$93^+ \longrightarrow 91^+ + 2$

Fraction 6

Results

Gas-liquid chromatography: the peak corresponding to this fraction was small and not well resolved from peak 5. It had a retention time of 6.1 mins. and represented 0.97% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.7	30	4.5	47	12.3
13	0.7	31	27.4	49	0.7
14	2.6	38	6.1	50	6.5
15	10.6	39	62.9	51	13.9
17	0.7	40	11.3	52	8.1
18	1.0	41	85.5	53	51.0
26	10.6	42	21.6	54	14.8
27	85.5	43	80.6	55	72.9
28	10.0	44	20.0	56	7.1
29	71.9	45	12.3	57	40.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
58	48.1	82	38.1	109	4.8
59	5.8	83	21.9	110	11.3
60	0.3	84	2.9	111	19.0
62	1.0	85	11.3	112	7.7
63	3.2	86	16.1	113	7.7
64	0.7	87	1.6	114	1.6
65	10.6	88	0.3	115	1.0
66	6.8	89	0.3	121	0.3
67	100.0	91	6.8	127	0.7
68	16.1	92	2.6	128	1.6
69	11.3	93	22.3	129	9.0
70	11.0	94	4.8	130	1.0
71	21.3	95	28.7	131	0.5
72	5.5	96	4.2	137	0.5
73	1.0	97	3.5	138	5.8
74	0.7	98	1.3	139	0.3
75	2.6	99	4.2	141	29.0
77	11.6	100	0.65	142	3.2
78	2.2	101	4.5	143	0.7
79	17.7	102	0.16	155	1.0
80	4.5	103	1.6	156	0.7
81	96.1	104	0.3		

M(156) = 0.7

Base peak: at m/e 67

Molecular formula: $C_{10}H_{20}O$

Double bond equivalents: one

Metastable ions: the same as Fraction 4

Discussion and conclusions

The molecular ion of these components seems to be m/e 156 as it is the highest mass in the spectra (112) and the following fragment ions may be logically derived from it (114):

$156[M]^{+\cdot}$ ⟶ m/e 141 -15 ($CH_3\cdot$)

⟶ m/e 129 -27 ($CH_2{=}CH\cdot$)

⟶ m/e 138 -18 (H_2O)

⟶ m/e 110 -46 (H_2O and $CH_2{=}CH_2$, CH_3CH_2OH)

The elemental composition of the molecular ion could not be derived from the heavy isotopes, but the elemental composition of the fragment ions m/e 141 and m/e 129 could be calculated as $C_9H_{17}O$ and $C_8H_{17}O$ respectively. The presence of oxygen could be deduced from the strong peak at m/e 31 and the smaller but distinct peaks at m/e 45 and 59 (126). By the addition of the lost fragments $CH_3\cdot$ and $CH_2{=}CH\cdot$ to the fragment ions m/e 141 and m/e 129 respectively, the molecular formula would be $C_{10}H_{20}O$. The other possibility is $C_{10}H_4O_2$ which is highly unsaturated. The general appearance of the spectra indicated the presence of aliphatic hydrocarbon moiety and thus rules out the molecular formula $C_{10}H_4O_2$.

The prominent peaks at m/e 27, 41 and 55 indicated the presence of olefinic or cyclic structure (165). The peaks at m/e 67, 81 and 95 suggested dienes, alkynes or cycloalkenes (166). A base peak at m/e 67 with the formula C_5H_7 may be derived from alkynes, alkadienes, cycloalkenes or bicyclic hydrocarbon (167).

The intensity of the molecular ion excludes any cyclic structure (161), and since there is only one double bond equivalent the possibilities are narrowed down to either unsaturated alcohol or ether. The presence of a large peak at m/e 31 favors this conclusion but can not be of much help in deciding if it is a primary alcohol or ether (126).

Beynon, Saunders and Williams (168) suggest that the best method to distinguish between alcohols and ethers is the pattern of peaks at the lower mass numbers which is much similar to the paraffinic pattern than the olefinic pattern in case of ethers, and that the region of C_2, C_3 and C_4 for primary alcohols is similar to the corresponding olefins (126). If these rules are extended then in this case the original compound had olefinic nature and acquired a diolefinic nature (series of fragment ions 67, 81 and 95). Thus a primary unsaturated alcohol is more probable than an unsaturated ether.

The peaks at m/e 141,129 and 113 which correspond to the loss of a $CH_3\cdot$, $CH_2{=}CH\cdot$, and $C_3H_7\cdot$ or ($CH_3\cdot$ and $CH_2{=}CH_2$) (120) suggest branching (169). The mass spectra of branched primary alcohols are considered to be the most difficult to interpret (170). Only preliminary attempt was made to collect information about the structure.

The presence of appropriate metastable ions substantiated some of the following sequences:

$C_{10}H_{20}O[M]^{\ddagger}$ $\xrightarrow{-(H_2O,\ CH_2=CH_2)}$ $[C_8H_{14}]^{\ddagger}$

m/e 156 m/e 110

$\xrightarrow{-CH_3\cdot}$ m/e 141 $\xrightarrow{-CH_2=CH_2}$ m/e 113

$\xrightarrow{-H_2O}$ m/e 138 $\xrightarrow{-CH\equiv CH}$ m/e 112

$\xrightarrow{-CH_2=CH\cdot}$ m/e 129 $\xrightarrow{-H_2O}$ m/e 111

$[C_8H_{14}]^{\ddagger}$ $\xrightarrow{-CH_3\cdot}$ m/e 95 $\xrightarrow{-2H\cdot}$ m/e 93 $\xrightarrow[*]{-2H\cdot}$ m/e 91

m/e 110

$\xrightarrow[*]{-CH_3CH_2\cdot}$ m/e 81 $\xrightarrow{-CH\equiv CH}$ m/e 55

$\xrightarrow[*]{-CH_2=CH_2}$ m/e 82 $\xrightarrow[*]{-CH_3\cdot}$ m/e 67 $\xrightarrow[*]{-CH\equiv CH}$ m/e 41

$\xrightarrow{-CH_2=CH\cdot}$ m/e 83 $\xrightarrow[*]{-CH_2=CH_2}$ m/e 55

The peaks at m/e 138 and m/e 81 in the spectrum of Fraction 6 were larger than the corresponding ones in the spectrum of Fraction 4. It seems that the isomer in Fraction 6 tends to lose water more readily. The loss of an ethyl radical from the fragment ion 110 seems to be more facile in the same isomer.

The presence of branched unsaturated primary alcohols of the formula $C_{10}H_{20}O$ has been reported in some volatile oils (171).

The ten most intense peaks of these two components were compared with reference compounds having the same

molecular formula (172), but they could not be identified.

In conclusion, Fractions 4 and 6 contain most probably two isomeric unsaturated branched primary alcohols of the formula $C_{10}H_{20}O$.

Fractions 5, pre 7, and 8

The mass spectra of these fractions were almost identical with the difference of 14 mass units between the highest peak of each, suggesting a homologous series.

Fraction 5

Results

Gas-liquid chromatography: this fraction had a small peak, a retention time of 5.6 mins., and represented 1.74% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	29	38.8	46	1.7
13	0.3	30	2.0	47	30.0
14	1.2	31	15.8	48	0.3
15	3.7	39	14.2	50	0.5
16	0.2	40	2.8	51	1.2
17	0.2	41	29.2	52	0.8
19	1.5	42	7.5	53	3.7
26	4.3	43	25.0	54	1.5
27	34.2	44	12.0	55	27.7
28	18.3	45	9.0	56	4.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
57	100.0	77	0.7	95	0.7
58	6.3	79	0.9	99	3.0
59	3.5	80	0.2	100	0.3
60	0.3	81	2.3	101	1.0
61	1.0	82	2.5	103	43.2
67	5.1	83	18.0	104	2.3
69	2.7	84	1.5	105	0.3
70	3.0	85	46.5	111	0.5
71	5.2	86	3.2	113	0.5
72	5.5	87	0.3	117	0.5
73	1.4	89	0.2	128	9.8
74	0.2	91	0.2	129	21.7
75	19.8	92	0.2	130	1.7
76	0.7	93	0.3	131	0.3

M(130) = 1.7

Base peak: at m/e 57

Molecular formula: $C_7H_{14}O_2$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
14.8	$57^+ \longrightarrow 29^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
54.5	$103^+ \longrightarrow 75^+ + 28$

Fraction pre 7

Results

Gas-liquid chromatography: the peak corresponding to this fraction was very small. It was augmented by p-cymene.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	48	0.3	71	5.6
13	0.5	50	0.3	72	5.6
14	1.3	51	1.0	73	1.0
15	2.8	52	0.5	74	0.3
17	0.5	53	3.3	76	0.5
18	1.3	54	2.6	77	0.5
19	1.3	55	32.3	79	0.8
26	3.8	56	5.6	80	0.3
27	32.1	57	100.0	81	5.1
29	54.6	58	6.2	82	1.5
30	2.1	59	3.6	83	0.5
31	14.4	61	0.5	85	56.2
39	13.3	62	0.3	86	3.6
40	2.6	63	0.3	87	0.3
41	34.6	64	0.3	91	0.3
42	10.3	65	0.8	93	0.3
43	28.2	66	1.0	95	1.3
44	10.8	67	4.4	96	1.8
45	7.9	68	5.4	97	10.8
46	1.3	69	6.9	98	1.3
47	25.6	70	4.6	99	6.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
100	0.5	110	0.1	127	0.3
101	0.3	112	0.1	131	0.5
102	2.6	113	2.3	141	0.5
103	48.5	114	0.4	142	9.0
104	2.6	115	0.5	143	16.4
105	0.3	119	0.5	144	1.5

M(144) = 1.5

Base peak: at m/e 57

Molecular formula: $C_8H_{16}O_2$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
14.8	$57^+ \longrightarrow 29^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
54.7	$103^+ \longrightarrow 75^+ + 28$
65.8	$143^+ \longrightarrow 97^+ + 46$

Fraction 8

Results

Gas-liquid chromatography: this fraction had a very small peak, a retention time of 8.8 mins., and represented 0.56% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	13	0.3	14	1.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
15	2.8	55	24.3	84	1.4
16	0.2	56	7.8	85	56.5
17	0.3	57	100.0	86	3.7
18	0.8	58	3.7	87	0.3
19	1.0	59	3.5	89	0.2
26	3.5	60	0.2	95	2.3
27	29.2	61	0.3	99	4.0
28	1.8	63	0.3	103	41.5
29	50.5	64	0.2	104	2.3
30	1.5	65	0.9	105	0.3
31	12.5	66	1.4	110	1.8
39	12.5	67	7.2	111	2.3
40	2.5	68	5.7	112	0.3
41	34.2	69	18.0	113	4.0
42	9.5	70	4.2	114	0.5
43	30.0	71	5.0	115	0.2
44	8.7	72	5.0	127	1.7
45	6.3	73	1.2	128	0.2
46	1.0	74	0.2	129	0.3
47	20.0	75	14.8	131	0.3
48	0.2	76	0.3	141	0.3
49	0.1	77	0.5	144	0.3
50	0.3	79	1.0	156	8.3
51	0.9	80	0.2	157	11.7
52	0.5	81	3.8	158	1.1
53	4.2	82	5.5	159	0.2
54	2.8	83	3.2		

M(158) = 1.1

Base peak: at m/e 57

Molecular formula: $C_9H_{18}O_2$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
14.8	$57^+ \longrightarrow 29^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
54.7	$103^+ \longrightarrow 75^+ + 28$
78.6	$157^+ \longrightarrow 111^+ + 46$

Discussion and conclusions

These three homologous compounds had identical mass spectra below the fragment ion m/e 103. The molecular ion of component 5 appears to be m/e 130, while that of pre 7 and 8, m/e 144 and 158. The fragment ion m/e 103 seems to be derived from the molecular ions by the loss of a vinyl radical and its higher homologs as follows:

130 $\xrightarrow{-27}$ m/e 103 (loss of $CH_2{=}CH\cdot$)

144 $\xrightarrow{-41}$ m/e 103 (loss of $CH_2{=}CHCH_2\cdot$)

158 $\xrightarrow{-55}$ m/e 103 (loss of $CH_2{=}C(CH_3)\cdot$)

If the first homolog was identified the remaining two could be constructed by the addition of the methylene group to $R\text{-}CH{=}CH_2$, where R has the mass of 103. The retention data originally suggested p-cymene for component 5, but the mass spectrum ruled that out (173).

In the mass spectrum of component 5 the intensity of

the molecular ion suggested an ether (139). The general appearance of the mass spectrum at the low mass end and the distinct peaks at m/e 31 and 45 also suggested an ether. Large peaks at m/e 47, 75 and 103 indicated the presence of two oxygen atoms.

The molecular formula was deduced from appendices of common fragment ions (118) and common fragments lost (120). The two most likely structures for component 5 are:

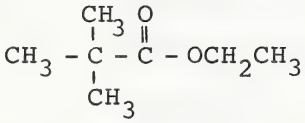
$CH_2 = CH - CH(OCH_2CH_3)_2$ or $C_4H_9\overset{\overset{O}{\|}}{C} - OCH_2CH_3$

Subsequently the structures for pre 7 and 8 would be:

$CH_2 = CH - CH_2 - CH(OCH_2CH_3)_2$ or $C_4H_9\overset{\overset{O}{\|}}{C} - OC_3H_7$

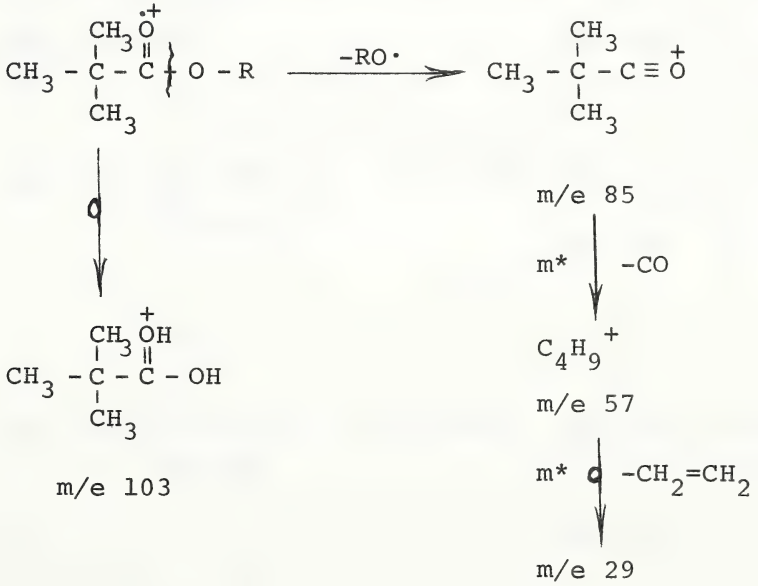
$CH_2 = \overset{\overset{CH_3}{|}}{C} - CH_2 - CH(OCH_2CH_3)_2$ or $C_4H_9\overset{\overset{O}{\|}}{C} - OC_4H_9$

The compilation data was consulted for comparison with reference compounds having similar molecular weights. Component 5 was compared with seventeen possible esters (174) but none matched. The ethyl esters of normal and isovaleric acids, although partially similar, had a peak at m/e 88 as the third most intense peak which could be derived from a McLafferty rearrangement and which was missing in the mass spectrum of component 5. The possible ethyl ester which would not allow such rearrangement is:



The base peak at m/e 57 which has the calculated composition C_4H_9 favors the above structure (175, 176). Component pre 7 was compared with thirteen possible esters (177, 178) but none of them fitted exactly. Component 8 was compared with seventeen esters (179, 180). The two esters, n-butyl isovalerate and isobutyl isovalerate, although similar, had rearrangement peaks at m/e 56 and 60 which were negligible in the mass spectrum of component 8.

The expected fragmentation pathway of the proposed structures for these esters would be (181, 182):



where R = CH_3CH_2 component 5

= C_3H_7 component pre 7

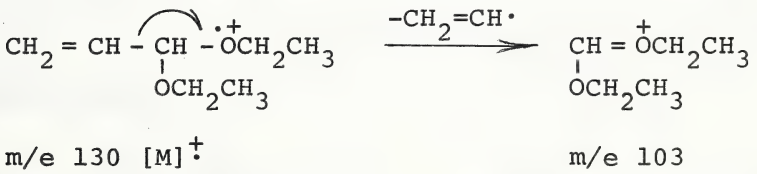
= C_4H_9 component 8

These structures do not account for the prominent peaks at m/e 47, 75 and M-1. Ether structures can explain these ions.

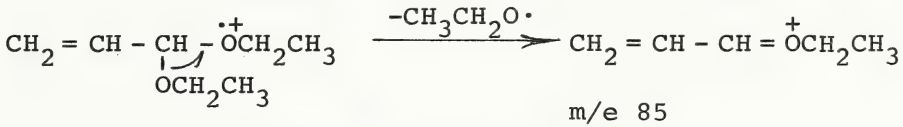
The ten most intense peaks of 1,1-diethoxy-3-methyl-3-butene (180) were very similar to the peaks of component 8. However the intensities of the peaks were different.

The fragmentation pathways of the unsaturated diethoxy compounds, which can be treated as acetals, are expected to be as follows (183, 184):

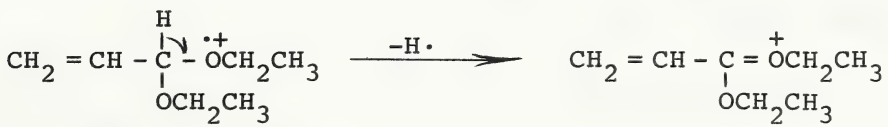
Component 5



m/e 130 $[M]^{+\cdot}$ m/e 103

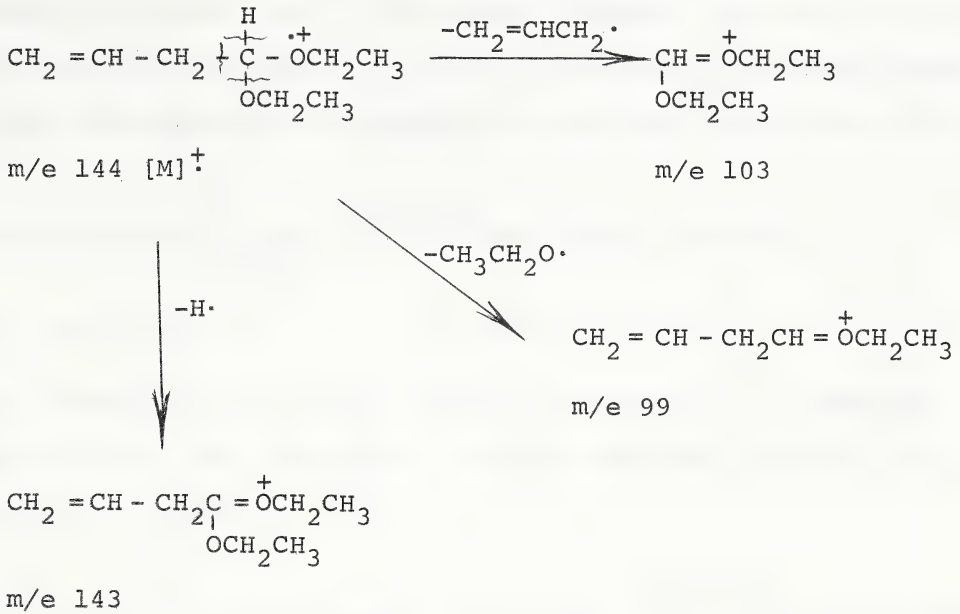


m/e 85

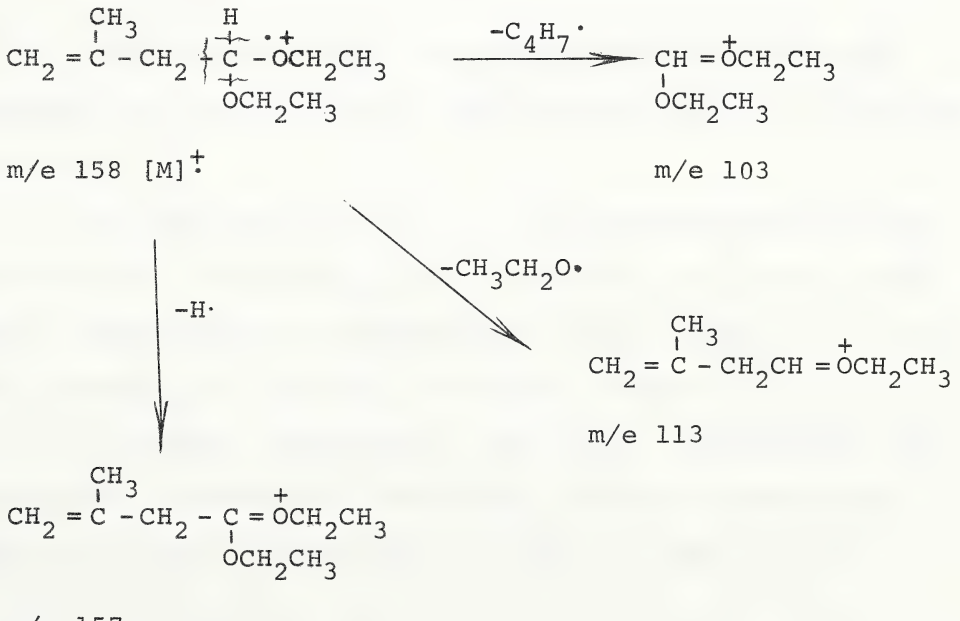


m/e 129

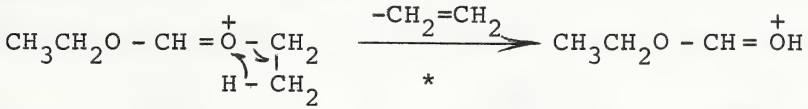
Component pre 7



Component 8

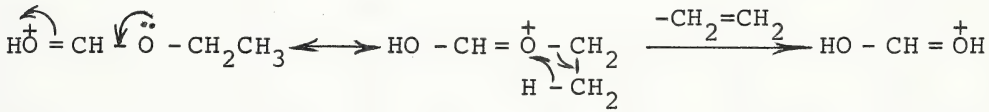


All these cleavages are β to the oxygen atom; a fragmentation common to ethers (140). The common fragment ion m/e 103 may be considered as the key to most of the other prominent peaks. It may decompose by α-cleavage with hydrogen migration (184):



m/e 103 m/e 75

The fragment ion m/e 75 by another α-cleavage with hydrogen migration and the release of a stable ethylene molecule will give m/e 47 (184):



m/e 75 m/e 47

In the three spectra there are peaks at M-2 which may be accounted for by the loss of two hydrogen atoms or a hydrogen molecule.

The base peak at m/e 57 in the three spectra and the intense peaks at m/e 85 in the spectra of pre 7 and 8 could not be accounted for satisfactorily by the acetal structures. This suggests the presence of another component.

In summary, Fractions 5, pre 7 and 8 may contain as their major components a homologous series of esters and acetals of the formulas $C_7H_{14}O_2$, $C_8H_{16}O_2$, and $C_9H_{18}O_2$.

Fraction 7

Results

Gas-liquid chromatography: this fraction had a sharp large symmetric peak, a retention time of 7.96 mins., and represented 1.64% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	44	64.6	70	4.5
13	0.2	45	17.5	71	8.9
14	1.1	46	0.9	72	5.5
15	4.5	47	1.1	73	1.8
16	0.2	50	1.3	74	0.7
17	0.2	51	2.5	75	0.7
18	0.2	52	1.3	77	0.7
19	0.4	53	8.4	79	1.4
26	4.5	54	7.9	80	0.7
27	60.2	55	54.6	81	17.5
28	7.3	56	49.5	82	17.5
29	77.3	57	59.8	83	3.4
30	2.1	58	7.9	84	35.5
31	4.3	59	1.6	85	15.9
38	1.8	63	0.5	86	2.1
39	40.2	65	1.6	87	0.9
40	7.3	66	3.0	91	0.2
41	99.1	67	15.9	93	0.4
42	42.3	68	17.7	95	5.4
43	100.0	69	21.1	96	0.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
97	0.7	110	7.3	129	0.2
98	0.4	111	0.9	131	0.4
99	2.3	112	0.2	141	0.2
100	7.0	113	0.5	142	0.3
101	1.3	115	0.9	143	0.5
103	1.1	117	0.3	144	0.2
104	0.2	126	0.4	147	0.4
105	0.2	127	0.5	152	0.3
109	0.9	128	0.5	156	0.4

M(128) = 0.5

Base peak: at m/e 43

Molecular formula: $C_8H_{16}O$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
29.5	$57^+ \longrightarrow 41^+ + 16$
59.7	$110^+ \longrightarrow 81^+ + 29$
67.3	$100^+ \longrightarrow 82^+ + 18$
56.6	$84^+ \longrightarrow 69^+ + 15$
37.3	$84^+ \longrightarrow 56^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
21.7	$85^+ \longrightarrow 43^+ + 42$
25.1	$29^+ \longrightarrow 27^+ + 2$

Discussion and conclusions

The small peak at m/e 128 is most probably due to the molecular ion because of the following logical fragment

losses:

m/e 128 $[M]^{+\cdot}$	⟶ m/e 127	- 1	(H·)
	⟶ m/e 110	-18	(H_2O)
	⟶ m/e 100	-28	(CO, or $CH_2{=}CH_2$)
	⟶ m/e 99	-29	(HCO·, or C_2H_5·)
	⟶ m/e 85	-43	(C_3H_7·, or CH_3CO·)
	⟶ m/e 84	-44	($CH_2{=}C(=O)-H + H$)

The molecular formula $C_8H_{16}O$ was deduced by tracing the homologous series of ions. The presence of oxygen was indicated by the peaks at m/e 31, and 45.

At the low mass end of the spectrum the general appearance of regular groups of peaks 14 mass units apart, each having the species C_nH_{2n+1} as the most abundant ion, indicated the presence of saturated hydrocarbon moiety (185).

The presence of prominent peaks at M-18 and M-28, and strong peaks at M-44 and m/e 44 suggested an aldehyde (186).

The intense peak at m/e 44, the absence of large peaks at m/e 58 or 72 (187), and the large M-44 peak (188) excluded α-substitution on an aldehyde. The following structure, which could account for the intense peaks in the spectrum through the fragmentation pathway common to aliphatic saturated aldehydes (189), was proposed for component 7:

$$C_6H_{13}-CH_2-C(=O)-H$$

Comparison of the mass spectrum of component 7 with a compilation of reference compounds suggested n-octanal (190). However mass spectra of branched aldehydes of the same molecular weight were not available for comparison.

Caprylaldehyde or n-octanal has been reported present in the ethereal oil of Citrus medica (191), Lavandula delphinensis (157), Xanthoxylum rhetsa (192), and possibly in lemon oil (155).

Fraction 9

Results

Gas-liquid chromatography: this fraction had a small peak which was not well resolved from peak 10. It had a retention time of 8.75 mins., and represented 0.63% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	39	31.0	50	2.4
13	0.5	40	6.7	51	3.8
15	10.0	41	78.6	52	2.4
17	1.4	42	16.2	53	11.0
18	8.3	43	100.0	54	3.8
26	2.9	44	8.6	55	50.5
27	31.0	45	5.7	56	18.6
29	34.8	46	0.1	57	35.7
30	1.4	47	3.8	58	13.8
31	5.7	48	0.5	59	8.6
32	1.4	49	0.5	60	1.9

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
61	3.3	85	14.8	112	1.9
62	0.5	86	1.0	113	1.9
63	1.4	87	1.3	114	1.0
64	0.5	88	1.4	115	4.3
65	2.4	91	1.9	116	1.0
66	2.9	93	5.7	117	1.0
67	14.3	95	2.4	119	1.0
68	12.4	96	1.4	121	0.5
69	32.4	97	2.4	126	4.8
70	9.0	98	2.9	127	4.3
71	11.0	99	3.3	129	1.4
72	1.9	100	0.5	131	1.0
73	2.9	101	2.9	139	0.2
74	32.9	102	0.5	141	0.5
75	5.2	103	8.1	142	0.5
77	2.4	104	1.0	143	0.5
79	1.9	105	1.9	145	0.5
80	1.0	107	1.0	156	1.9
81	6.2	108	11.4	157	2.4
82	4.3	109	1.9	158	0.5
83	11.0	110	1.0	161	1.0
84	3.8	111	10.0	162	0.5

M(158) = 0.5

Base peak: at m/e 43

Molecular formula: $C_9H_{18}O_2$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
80.3	$127^+ \longrightarrow 101^+ + 26$
92.6	$143^+ \longrightarrow 115^+ + 28$
54.6	$126^+ \longrightarrow 83^+ + 43$
36.5	$83^+ \longrightarrow 55^+ + 28$
24.4	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions:

This fraction seems to contain more than one component because of the appearance of peaks with mass differences which can not possibly be due to easily eliminated fragments (148).

The molecular formula of one of the components (m/e 158) was deduced by tracing the homologous series of ions and the fragmentation pattern. The following logical mass differences to the neighboring major ions verify 158 to be the molecular ion:

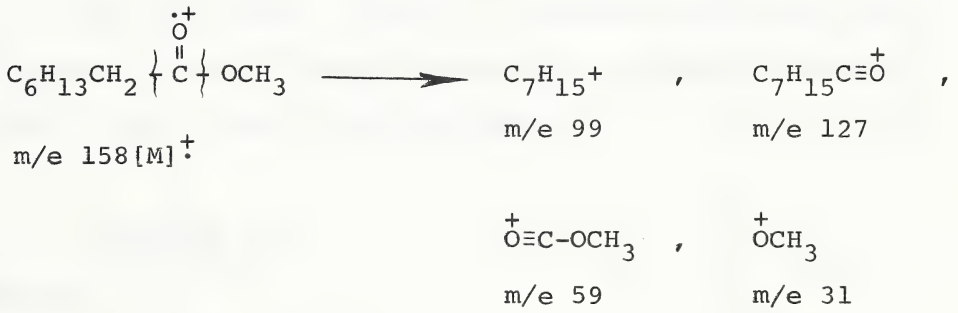
m/e 158$[M]^{+\cdot}$	$\longrightarrow$ m/e 129	-29	($C_2H_5\cdot$)
	$\longrightarrow$ m/e 127	-31	($CH_3O\cdot$)
	$\longrightarrow$ m/e 126	-32	(CH_3OH)
	$\longrightarrow$ m/e 115	-43	($C_3H_7\cdot$)

The intense peak at m/e 74 suggested a methyl ester of a fatty acid (193, 194). The following structure was proposed:

$$C_6H_{13}CH_2\overset{\overset{O}{\|}}{C}OCH_3$$

As this fraction is not composed solely of this methyl ester, it was difficult to check the structure by comparison with reference compounds. However the ten most intense peaks of methyl octanoate (195) also existed in the mass spectrum of 9, but m/e 87 which was the second most intense peak was very weak in the mass spectrum of 9.

The proposed structure may fragment by α-cleavage to yield the fragment ions (196):

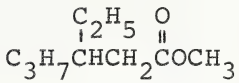


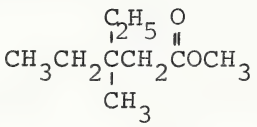
The low abundance of the ions m/e 31, 99 and [M-31] are consistent with long straight chain methyl esters (196). Component 9 can also fragment by β-cleavage with the transfer of a γ-hydrogen atom to yield m/e 74, which is the base peak in long straight chain methyl esters (197).

The following observations suggested that the hydrocarbon chain may be branched: the peak at m/e 85 was more intense than that at m/e 71 (198), the loss of C_2H_5 and C_3H_7 radicals from the molecular ion (199), the elimination of a molecule of methanol from the molecular ion (200), the fragment ion resulting from the elimination of methanol was shown by a suitable metastable to lose 43 mass units which

may be a C_3H_7 radical (201), and the absence of an intense peak at m/e 87 corresponding to $[(CH_2)_2COOCH_3]^+$ which is favored in long straight chain methyl esters (202).

The structure of this methyl ester may be one of the following:





In conclusion, fraction 9 contains more than one component. One of these components is probably a methyl ester of the formula $C_6H_{13}CH_2COOCH_3$.

Fraction 10

Results

Gas-liquid chromatography: this fraction had a small blunt peak, a retention time of 10.5 mins., and represented 1.17% of the oil. The retention data suggested methyl heptenone.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	31	2.4	46	0.6
15	5.0	39	15.9	47	1.2
17	0.9	40	2.9	50	0.3
18	0.6	41	39.7	51	1.2
26	1.7	42	11.5	52	0.6
27	22.1	43	100.0	53	3.5
29	27.4	44	6.8	54	2.1
30	0.6	45	3.5	55	19.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
56	9.7	83	2.6	109	0.9
57	33.8	84	3.2	110	0.6
58	46.8	85	7.1	111	1.2
59	17.4	86	0.9	112	0.3
60	0.9	87	0.9	113	1.2
67	3.9	88	1.2	114	0.8
68	3.8	91	2.1	115	0.8
69	7.4	92	1.5	117	0.6
70	4.7	93	1.2	119	0.3
71	20.3	95	2.6	124	0.9
72	2.1	98	2.6	126	0.3
73	0.9	99	2.4	127	2.4
74	1.5	100	0.9	128	0.6
75	1.7	101	0.9	129	0.6
78	0.6	103	1.8	131	0.3
79	0.3	104	0.3	133	0.3
80	0.3	105	0.6	142	4.7
81	4.1	107	0.3	143	0.6
82	4.7	108	0.6	145	0.6
				147	1.5

M(142) = 4.7

Base peak: at m/e 43

Molecular formula: $C_9H_{18}O$

Double bond equivalents: one

Discussion and conclusions

The molecular ion (m/e 142) is distinct. The general

appearance of the mass spectrum looked like a saturated straight chain hydrocarbon (203), except for the second most intense peak at m/e 58 which indicated specific rearrangement (204). The presence of oxygen was indicated by a small peak at m/e 31, 45, and a large one at m/e 59. The molecular formula $C_9H_{18}O$ was deduced by tracing the homologous series of ions.

The presence of oxygen, the base peak at m/e 43 and the large peaks at m/e 57, 71, which are isobaric with paraffin hydrocarbons (125), together with the rearrangement peak at m/e 58 (205, 206) suggested a ketone. The base peak at m/e 43, the intense rearrangement peak at m/e 58 (207), and the distinct M-15 peak, suggested a methyl ketone.

The proposed structure below accounts for almost all the peaks in the mass spectrum through the fragmentation pathway common to aliphatic ketones (207, 183).

$$C_3H_7CH_2CH_2CH_2CH_2\overset{\overset{O}{\|}}{C}CH_3$$

Comparison with reference compounds also suggested 2-nonanone (208).

The retention data previously suggested methyl heptenone, but this was ruled out from the general appearance of the mass spectrum excluding any unsaturation, and the absence of a peak corresponding to the molecular weight of methyl heptenone.

The presence of 2-nonanone has been reported in the

essential oil of Boronia ledifolia (209), and Ruta chalepensis (210).

Thus it was concluded that Fraction 10 contains one component which is most probably 2-nonanone.

Fraction 12

Results

Gas-liquid chromatography: this fraction had a small peak, which was not well resolved from peak 13, a retention time of 12.5 mins., and represented 0.52% of the oil. The peak was augmented by fenchone.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.6	42	21.2	58	3.5
14	1.8	43	100.0	59	2.4
15	6.5	44	4.7	61	8.8
17	1.8	45	4.1	63	0.6
18	5.4	46	0.6	64	0.6
26	2.4	47	2.4	65	1.8
27	30.6	50	1.2	66	1.8
29	31.2	51	1.8	67	8.8
30	0.6	52	1.2	68	9.4
31	7.6	53	7.6	69	24.7
38	1.2	54	5.9	70	23.5
39	22.9	55	68.2	71	10.0
40	4.7	56	28.2	72	2.9
41	35.0	57	26.5	73	4.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
74	0.6	95	3.5	112	5.9
75	1.8	96	1.9	113	1.8
77	1.8	97	7.6	114	0.6
79	2.4	98	2.4	115	1.2
80	0.6	99	1.8	116	1.2
81	5.3	101	1.8	117	1.2
82	4.7	103	5.4	119	1.2
83	12.9	105	1.2	121	0.6
84	11.8	107	0.6	124	0.3
85	8.8	108	1.2	125	1.7
86	1.9	109	1.2	126	3.5
87	1.9	110	5.9	127	2.4
91	0.6	111	15.3	129	0.6

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
27.2	$111^+ \longrightarrow 55^+ + 56$
61.7	$112^+ \longrightarrow 83^+ + 29$
36.4	$83^+ \longrightarrow 55^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$
37.3	$84^+ \longrightarrow 56^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
62.4	$116^+ \longrightarrow 85^+ + 31$

Discussion and conclusions

The retention data of this fraction suggested fenchone. The published mass spectrum of fenchone (211) did not match

that of Fraction 12.

The molecular ion in the mass spectrum of this fraction seems to be missing as there are many peaks at the high mass end which can not be derived from the highest mass ion.

The general appearance of the mass spectrum indicated the presence of aliphatic hydrocarbon moiety. The number of carbon atoms could be traced up to C_8. The series of prominent peaks of odd-electron ions could be traced from m/e 42 to m/e 126. The presence of oxygen was deduced from the peaks at m/e 31, 73, 101 and 103. The distinct peak at m/e 61 suggested acetate (130).

No conclusions could be made about the components of this fraction.

Fraction 13

Results

Gas-liquid chromatography: this fraction had a small blunt peak, a retention time of 12.95 mins., and represented 0.78% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.7	26	5.0	39	46.4
13	0.7	27	53.6	40	7.9
14	1.4	29	66.4	41	100.0
15	3.6	30	1.4	42	40.7
17	0.7	31	7.9	43	92.9
18	3.6	38	2.1	44	5.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
45	7.1	74	2.9	107	1.4
46	0.7	75	2.9	108	2.8
47	0.7	77	2.9	109	2.1
50	2.1	79	5.7	110	2.1
51	6.4	81	11.4	111	5.7
53	13.6	82	11.4	112	2.8
54	5.7	83	20.7	113	2.1
55	75.7	84	8.6	114	0.7
56	20.0	85	6.4	115	3.6
57	38.6	86	0.7	116	2.1
58	7.1	87	5.0	117	26.4
59	12.1	89	1.4	118	2.1
60	7.9	91	2.9	119	0.7
61	4.3	93	7.1	124	2.8
62	0.7	94	2.9	125	1.4
63	1.4	95	10.0	126	0.7
64	0.7	96	2.1	127	1.7
65	4.3	97	6.4	128	0.4
66	2.9	98	3.6	129	1.4
67	26.4	99	27.9	130	2.1
68	13.6	100	2.9	131	1.4
69	26.4	101	2.1	132	0.7
70	70.7	102	0.7	133	0.7
71	28.6	103	1.4	137	1.4
72	4.3	104	0.7	140	0.4
73	8.6	105	0.7	141	1.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
142	0.7	145	1.4	147	1.7
143	1.4	146	0.4	148	0.4

M(130) = 2.1

Base peak: at m/e 41

Molecular formula: $C_7H_{14}O_2$

Double bond equivalents: one

Metastable ions:

Diffuse peaks	Possible break-down reactions
38.2	$85^+ \longrightarrow 57^+ + 28$
43.2	$70^+ \longrightarrow 55^+ + 15$
56.6	$84^+ \longrightarrow 69^+ + 15$

Discussion and conclusions

The presence of several unrelated ions at the high mass end of the spectrum indicated that this fraction contains more than one component. The general appearance of the mass spectrum pointed to the presence of aliphatic hydrocarbon containing oxygen.

The intense peak at m/e 70 suggested amyl ester (212, 213), while m/e 60 and 61 suggested acetate (130). The published mass spectral data of normal and isoamyl acetate (174) are similar but not identical to those of Fraction 13. The variation may be attributed to the presence of other components.

The large peak at m/e 99 is not usual in the spectra of hydrocarbons and suggests $[RCO]^+$ (130) which corresponds to $[C_5H_{11}CO]^+$. This ion was assumed to be derived from

m/e 130 by the loss of 31 mass units, suggesting a methyl ester of the formula $C_5H_{11}COOCH_3$. This assumption was not supported by the presence of a strong peak at m/e 74 (197, 214) or m/e 88 (214). The large m/e 117 peak could not be accounted for.

In conclusion, Fraction 13 contains more than one component. One of the components may be isoamyl acetate.

Isoamyl alcohol has been reported to occur as such and as esters in the volatile oil of Eucalyptus globulus, E. aggregata, and in Roman chamomile (215).

Fraction 14

Results

Gas-liquid chromatography: this fraction had a small blunt peak, a retention time of 14.1 mins., and represented 1.04% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	30	1.0	44	6.7
13	0.5	31	9.5	45	6.2
14	2.4	37	0.5	46	0.5
17	2.9	38	1.0	47	1.4
18	9.5	39	23.8	48	0.5
19	1.0	40	4.8	49	0.5
26	3.3	41	59.5	50	1.9
27	32.4	42	13.8	51	2.4
29	33.8	43	100.0	52	1.9

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
53	12.4	84	4.8	111	8.1
54	5.2	85	13.8	112	4.3
55	47.1	86	2.9	113	2.9
56	15.2	87	1.4	114	2.4
57	32.4	88	1.0	115	1.4
58	17.6	89	0.5	116	0.5
59	27.6	91	2.9	117	1.4
60	1.0	92	0.5	119	3.3
64	1.0	93	10.0	121	1.4
65	2.9	94	7.6	122	1.0
66	2.4	95	10.5	123	0.5
67	17.0	96	3.8	124	0.5
68	13.8	97	8.1	125	1.9
69	20.0	98	2.9	126	1.6
70	12.4	99	15.7	127	2.9
71	23.8	100	2.4	128	1.9
72	7.1	101	4.8	129	0.5
73	1.9	102	0.5	131	0.5
74	0.5	103	1.0	133	0.5
75	2.4	104	0.5	135	1.0
77	2.9	105	1.0	137	1.4
79	7.1	106	0.5	141	0.5
80	0.5	107	1.9	145	0.5
81	16.7	108	1.4	151	0.5
82	6.7	109	3.8	152	0.5
83	14.3	110	2.9	155	1.9

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
156	0.5	161	1.0	170	1.9
157	1.0	167	0.5	171	0.5
160	0.5	168	0.5		

M(170) = 1.9

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
36.4	$83^+ \longrightarrow 55^+ + 28$
55.7	$83^+ \longrightarrow 68^+ + 15$
62.4	$113^+ \longrightarrow 84^+ + 29$
63.4	$114^+ \longrightarrow 85^+ + 29$
65.4	$138^+ \longrightarrow 95^+ + 43$

Discussion and conclusions

The highest mass ion in the spectrum of this fraction was m/e 170 from which m/e 155 is most probably derived by the loss of a methyl radical. The general appearance of the spectrum at the low mass end indicated oxygenated aliphatic hydrocarbon which was not identified.

The following fractions were shown by their mass spectra to contain large molecules having more than ten carbon atoms. Some of these are sesquiterpenes. They are grouped according to some common mass spectral features when possible. It was fully realized that identification of these complex structures by their mass spectra is very difficult (216, 217). At the present state of knowledge of fragmentation of sesquiterpenes it is impossible to reconstruct the molecule from the ion abundances (218). However certain correlations between the ions and the molecular structure can be derived. If the general class to which these components belong is known, argument by analogy may make identification possible (219, 220). For this reason the mass spectra of these fractions were compared with those of reference monoterpenes (211, 221, 222, 223, 224, 225) and sesquiterpenes (226, 227, 228, 229, 230).

Fractions 11, 15, 16, 17, 18, 19, 20, 21 and 31

The mass spectra of these fractions showed at the high mass end peaks at m/e 204. There were also peaks at m/e 189 and 161 most probably corresponding to the loss of 15 and 43 mass units from m/e 204.

The general appearance of the mass spectra pointed to the presence of alkyl hydrocarbon moieties with the regular repeating pattern of groups of peaks 14 mass units apart (203). The components of these fractions most probably are aliphatic. This was deduced either from the intensities of the molecular

ions (161), or from the infrared spectra, when available.

The large peaks at m/e 77, 79, 91, 105 and 119 indicated that these components tend to lose hydrogen atoms or molecules during fragmentation to form double bonds and aromatic rings (231).

The general appearance of the lower mass end of the spectra of Fractions 11 and 15 looked different from those of the rest of the group.

Fraction 11

Results

Gas-liquid chromatography: this fraction had a small peak, a retention time of 11.4 mins., and represented 0.78% of the essential oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	1.0	30	2.0	50	1.0
13	1.0	31	24.0	51	3.0
14	2.0	39	31.0	52	2.0
15	4.0	40	7.0	53	11.0
17	2.0	41	95.0	54	11.0
18	7.0	42	32.0	55	58.0
19	1.0	43	80.0	56	43.0
26	4.0	44	9.0	57	100.0
27	45.0	45	7.0	58	17.0
28	33.0	46	1.0	59	5.0
29	63.0	47	3.0	65	4.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
67	14.0	97	4.0	127	1.0
68	10.0	98	4.0	129	2.0
69	28.0	99	7.0	130	1.0
70	38.0	100	1.0	131	2.0
71	13.0	101	2.0	132	0.5
72	13.0	103	4.0	133	3.0
73	3.0	104	1.0	134	2.0
74	1.0	105	12.0	135	1.0
77	6.0	106	3.0	136	3.0
78	1.0	107	4.0	137	0.5
79	6.0	108	2.0	140	0.5
80	2.0	109	3.0	141	1.0
81	13.0	110	3.0	142	1.0
82	6.0	111	2.0	143	1.0
83	9.0	112	3.0	145	2.0
84	5.0	113	1.0	146	1.0
85	16.0	114	0.5	147	2.0
86	1.0	115	3.0	148	1.0
87	1.0	116	0.5	153	0.5
88	1.0	117	3.0	154	0.5
91	8.0	118	1.0	155	0.5
92	6.0	119	13.0	156	0.5
93	10.0	120	3.0	157	0.5
94	1.0	121	3.0	160	0.5
95	6.0	122	1.0	161	13.0
96	2.0	126	1.0	162	2.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
189	1.5	205	1.5	207	1.5
204	4.0	206	1.0		

M(204) = 4.0

Base peak: at m/e 57

Metastable ions:

Diffuse peaks	Possible break-down reactions
29.5	$161^+ \longrightarrow 69^+ + 92$
24.5	$69^+ \longrightarrow 41^+ + 28$
38.2	$85^+ \longrightarrow 57^+ + 28$
39.2	$83^+ \longrightarrow 57^+ + 26$
43.2	$70^+ \longrightarrow 55^+ + 15$

Discussion and conclusions

This fraction seems to contain more than one component because of the presence of peaks which can not be related to each other by reasonable fragmentation. One of these components may have a molecular weight of 204.

The homologous series of ions could be traced up to C_{12}. Peaks at m/e 31, 45 and 59 etc. indicated the presence of oxygen.

The base peak at m/e 57 is shown by a suitable metastable to be derived from m/e 85 and 83. The intense peak at m/e 70, which suggests amyl ester (213), is shown by a metastable to lose a methyl radical yielding m/e 55. The large m/e 85 peak may be partly due to $C_4H_9C{=}O$ ion.

The major component of this fraction seems to be aliphatic oxygenated hydrocarbon with more than ten carbon atoms.

Fraction 15

Results

Gas-liquid chromatography: this fraction had a small blunt peak, a retention time of 14.9 mins., and represented 0.82% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.8	45	6.8	67	30.0
13	0.8	46	0.8	68	16.4
14	2.4	47	2.0	69	28.4
15	8.8	48	0.4	70	13.2
16	0.4	49	0.8	71	17.2
17	1.6	50	2.0	72	3.6
19	0.8	51	4.4	73	3.2
20	0.4	52	2.4	74	0.4
26	4.4	53	17.2	75	1.6
27	79.2	54	11.6	77	10.0
29	76.4	55	71.6	78	1.2
30	0.8	56	22.0	79	10.8
31	10.8	57	79.2	80	4.4
38	1.6	58	4.0	81	26.4
39	32.0	59	3.2	82	11.2
40	7.2	62	0.8	83	18.8
41	82.0	63	1.6	84	8.4
42	16.0	64	0.4	85	10.8
43	100.0	65	5.2	86	2.0
44	8.0	66	4.0	87	0.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
88	0.4	117	1.2	155	0.8
89	0.8	118	0.8	156	0.4
91	6.8	119	4.4	157	0.8
92	2.4	120	0.8	159	0.4
93	34.8	121	3.2	161	2.0
94	4.0	122	0.8	162	0.6
95	10.4	123	0.8	163	0.6
96	3.6	124	1.6	164	0.8
97	5.2	125	1.6	166	0.6
99	4.0	126	1.2	167	0.4
100	1.2	127	2.8	168	0.6
101	2.0	128	0.8	169	0.4
103	4.8	129	1.6	171	0.6
104	1.2	131	0.4	173	0.6
105	2.8	133	0.8	175	0.4
106	0.8	136	2.0	179	0.6
107	3.2	137	0.4	180	0.4
108	1.6	138	0.8	181	0.6
109	5.2	139	1.2	182	0.4
110	5.2	141	1.6	183	0.6
111	24.8	143	0.4	184	0.8
112	3.6	144	0.4	185	0.8
113	1.2	145	0.8	187	0.4
114	0.8	147	0.4	188	0.4
115	0.8	151	0.8	189	0.6
116	0.4	154	0.4	190	0.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
191	0.4	202	0.4	206	0.6
193	1.0	203	0.6	207	1.6
194	0.4	204	1.0	208	0.8
201	0.4	205	0.6	209	0.4

M(204) = 1.0

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
29.5	$161^+ \longrightarrow 69^+ + 92$
24.4	$69^+ \longrightarrow 41^+ + 28$
37.2	$121^+ \longrightarrow 67^+ + 54$
63.7	$136^+ \longrightarrow 93^+ + 43$
89.8	$93^+ \longrightarrow 91^+ + 2$
56.7	$105^+ \longrightarrow 77^+ + 28$
38.3	$85^+ \longrightarrow 57^+ + 28$
39.2	$83^+ \longrightarrow 57^+ + 26$

Discussion and conclusions

The presence of several unrelated peaks at the high mass end of the spectrum indicated the presence of more than one component in this fraction.

The peaks at m/e 204, 189 and 161 suggested that one of the components has a molecular weight of 204. The small peaks below m/e 204, which can not be reasonably derived from it, indicated the presence of higher component, whose molecular ion is missing. The loss of 43 mass units from m/e 136, which was substantiated by a suitable metastable

ion, to give an intense peak at m/e 93 suggested a monoterpene hydrocarbon. The peak at m/e 136 may also be due to a rearrangement ion derived from a sesquiterpene hydrocarbon by the loss of an isoprene unit.

However the mass spectrum of this fraction was compared with those of seventeen monoterpene hydrocarbons (222), but no conclusions could be reached.

The presence of oxygen was indicated by the peaks at m/e 31, 45, 59 etc.

In summary, this fraction contains a mixture of components: one of these components may have a molecular weight of 204, a higher component, and may be a monoterpene hydrocarbon of the formula $C_{10}H_{16}$.

Fraction 16

Results

Gas-liquid chromatography: this fraction had a blunt peak, a retention time of 15.7 mins., and represented 1.43% of the oil. The peak was augmented by l-bornyl acetate.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	19	2.2	37	2.1
13	0.7	26	4.6	38	2.9
14	2.5	27	49.3	39	50.0
15	5.7	29	57.5	40	10.0
16	0.4	30	1.1	41	100.0
17	1.4	31	11.1	42	29.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
43	82.9	70	27.1	97	7.9
44	7.1	71	16.1	98	6.4
45	20.4	72	3.6	99	6.4
46	1.4	73	15.0	100	1.1
47	1.4	74	1.4	101	11.4
48	0.7	75	2.2	102	0.7
49	0.4	76	0.4	103	2.1
50	3.9	77	11.1	104	0.7
51	6.8	78	2.9	105	8.6
52	3.6	79	19.3	106	2.9
53	20.0	80	3.9	107	10.4
54	7.9	81	27.1	108	5.4
55	81.8	82	14.3	109	22.1
56	30.0	83	21.8	110	6.4
57	35.0	84	11.8	111	5.0
58	5.4	85	9.3	112	2.2
59	3.6	86	1.8	113	2.9
60	1.8	87	1.8	114	0.7
62	0.7	88	0.4	115	2.1
63	2.5	89	0.4	116	1.4
64	1.1	91	12.5	117	4.3
65	11.1	92	3.6	118	1.1
66	8.9	93	15.4	119	5.0
67	66.1	94	5.4	120	2.9
68	15.7	95	23.6	121	5.4
69	28.9	96	11.4	122	2.9

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
123	4.3	141	1.1	167	0.4
124	3.6	142	0.4	168	0.4
125	10.0	144	2.9	169	0.7
127	2.9	145	1.4	175	1.4
128	1.4	146	3.2	176	0.7
129	3.6	147	1.4	189	3.2
130	0.7	148	5.0	190	0.7
131	3.2	150	0.4	191	1.1
132	0.4	151	0.4	192	0.4
133	4.6	153	0.4	193	0.4
134	1.1	159	1.1	204	3.9
135	3.2	161	7.9	205	1.1
136	1.4	162	1.8	206	0.7
137	0.7	163	0.7	207	1.1
138	8.9	164	0.4	208	0.7
139	1.1	165	0.7	209	0.4
140	1.1	166	0.4		

M(204) = 3.9

Base peak: at m/e 41

Metastable ions:

Diffuse peaks	Possible break-down reactions
60.3	$204^+ \longrightarrow 111^+ + 93$
93.6	$204^+ \longrightarrow 138^+ + 66$
62.2	$111^+ \longrightarrow 83^+ + 28$
56.7	$105^+ \longrightarrow 77^+ + 28$
37.5	$121^+ \longrightarrow 67^+ + 54$
89.2	$93^+ \longrightarrow 91^+ + 2$
46.8	$91^+ \longrightarrow 65^+ + 26$
38.3	$85^+ \longrightarrow 57^+ + 28$
39.2	$83^+ \longrightarrow 57^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions

The retention data suggested l-bornyl acetate, but this was ruled out on comparison of the mass spectrum with that of bornyl acetate (224).

The general appearance of the spectrum looked like those of sesquiterpene hydrocarbons (230). The high mass end was similar to that of trans β-farnesene.

The peaks at m/e 205, and 162 were too high to be accounted for as only due to heavy isotopes. The peaks at m/e 191, 205 and 207 suggested the presence of a higher component than 204. They also indicated the probability that m/e 204 may be a fragment ion. The peaks at m/e 31, 45, 59, 73 and 101 indicated the presence of oxygen.

However m/e 204 may be explained as M-18 peak, while m/e 205 and 207 may correspond to M-17 and M-15 peaks. Thus

the missing molecular ion may be 222.

The large peak at m/e 101 may be due to $C_4H_9OC=O$ (232) which may lose CO to yield m/e 73 which is represented by a large peak. The large peaks at m/e 125 and 101 suggest esters of aliphatic dibasic carboxylic acid (233).

In conclusion, Fraction 16 may contain a sesquiterpene hydrocarbon, $C_{15}H_{24}$, as one of its components, or a sesquiterpene alcohol of the formula $C_{15}H_{25}OH$.

Echinopanacen, a sesquiterpene of the formula $C_{15}H_{24}$, and echinopanacol, a sesquiterpene alcohol of the formula $C_{15}H_{25}OH$, have been isolated from the same plant in 1927 in Japan (81).

Fraction 17

Results

Gas-liquid chromatography: this fraction had a rather large symmetric peak, a retention time of 17.4 mins., and represented 1.82% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
15	5.7	38	5.7	45	6.0
17	1.8	39	57.0	47	1.2
26	5.4	40	10.2	50	3.0
27	54.0	41	98.0	51	9.6
29	63.0	42	27.0	52	2.4
30	2.1	43	100.0	53	20.1
31	6.3	44	13.5	54	10.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
55	78.5	84	16.5	116	0.9
56	28.2	85	10.2	117	3.0
57	47.4	87	1.8	119	3.0
58	6.3	89	0.9	120	1.8
59	2.4	91	8.1	121	2.7
60	2.1	93	20.1	122	3.0
63	0.9	94	6.3	123	2.1
65	5.7	95	16.2	125	1.8
66	4.5	96	18.6	126	1.8
67	22.5	97	9.0	127	3.3
68	16.5	98	5.7	128	0.9
69	36.3	99	4.8	129	1.5
70	37.8	100	0.9	130	1.2
71	16.2	101	1.8	131	3.3
72	5.4	103	2.4	133	1.5
73	6.9	105	3.9	135	1.2
74	1.2	106	1.2	138	0.6
75	2.7	107	5.4	145	2.1
77	8.1	108	1.8	147	1.5
78	1.2	109	7.2	161	3.3
79	9.6	110	3.6	162	0.6
80	2.4	111	18.9	189	1.2
81	28.8	112	3.0	204	2.4
82	11.1	113	3.6	205	0.6
83	29.4	115	1.2	207	0.9

M(204) = 2.4

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
37.0	$121^+ \longrightarrow 67^+ + 54$
56.6	$105^+ \longrightarrow 77^+ + 28$
89.0	$93^+ \longrightarrow 91^+ + 2$
38.2	$85^+ \longrightarrow 57^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$
24.3	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions

At the high mass end of the spectrum the peaks were small. At the low mass end there were two intense peaks at m/e 70 and 96 corresponding to odd-electron ions.

Comparison of the mass spectrum of this fraction with those of reference compounds gave no clue about the structure.

Fraction 18

Results

Gas-liquid chromatography: this fraction had a small peak, a retention time of 18.1 mins., and represented 1.17% of the oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.8	16	0.8	20	0.4
13	0.8	17	1.9	25	0.4
15	4.2	19	0.4	26	3.5

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
27	45.4	59	2.7	89	1.5
29	46.5	63	2.3	90	0.8
30	1.1	64	0.8	91	39.6
31	5.4	65	11.9	92	12.3
37	0.8	66	5.0	93	46.9
38	1.5	67	36.5	94	12.3
39	38.5	68	15.0	95	18.8
40	8.5	69	35.0	96	7.3
41	100.0	70	10.4	97	11.5
42	15.8	71	21.2	98	2.3
43	75.8	72	1.9	99	7.7
44	5.8	73	4.2	100	1.5
45	6.2	74	0.4	101	1.5
46	0.8	75	2.3	102	1.1
47	0.8	77	27.7	103	3.8
48	0.4	78	4.6	104	2.7
49	0.8	79	28.8	105	40.4
50	2.7	80	7.3	106	30.4
51	7.3	81	40.8	107	30.0
52	3.8	82	14.6	108	9.6
53	24.6	83	19.6	109	15.8
54	7.3	84	5.8	110	6.2
55	72.7	85	10.4	111	9.6
56	16.5	86	2.7	112	2.7
57	41.5	87	1.1	113	3.5
58	9.2	88	0.4	114	1.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
115	4.6	141	1.5	167	0.4
116	1.9	142	1.1	168	0.4
117	6.2	143	1.1	169	0.8
118	2.7	144	0.4	170	0.4
119	18.4	145	4.2	171	0.4
120	7.3	146	1.1	173	0.4
121	7.7	147	9.6	174	0.4
122	8.8	148	14.2	175	2.7
123	28.5	149	1.9	176	2.3
124	4.2	150	1.1	177	0.8
125	7.7	151	1.5	178	0.4
126	1.1	152	1.1	179	0.4
127	7.3	153	1.5	180	0.4
128	3.5	154	0.4	181	0.8
129	2.7	155	1.1	182	0.4
130	1.9	156	0.8	183	0.4
131	6.5	157	1.1	184	0.4
132	2.3	158	0.8	187	1.9
133	17.3	159	3.5	188	0.4
134	5.0	160	1.1	189	12.7
135	3.8	161	11.2	190	1.9
136	2.7	162	6.5	191	0.8
137	1.5	163	3.8	192	0.4
138	2.3	164	0.8	193	0.4
139	0.4	165	1.1	194	0.4
140	0.8	166	0.4	196	0.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
197	0.4	201	0.4	205	5.4
198	0.4	202	2.3	206	0.6
199	0.4	203	0.8	207	1.1
200	0.4	204	34.6		

M(204) = 34.6

M+1(205) = 5.4

M+2(206) = 0.6

Isotope abundances:

m/e	% of M
204(M)	100.
205(M+1)	15.6
206(M+2)	1.7

Base peak: at m/e 41

Molecular formula: $C_{15}H_{24}$

Double bond equivalents: four

Metastable ions:

Diffuse peaks	Possible break-down reactions
93.6	$204^+ \longrightarrow 138^+ + 66$
63.6	$136^+ \longrightarrow 93^+ + 43$
66.5	$93^+ \longrightarrow 79^+ + 14$
37.1	$121^+ \longrightarrow 67^+ + 54$
25.6	$129^+ \longrightarrow 57^+ + 72$
58.3	$107^+ \longrightarrow 79^+ + 28$
69.6	$119^+ \longrightarrow 91^+ + 28$
89.0	$93^+ \longrightarrow 91^+ + 2$
38.2	$85^+ \longrightarrow 57^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions

The molecular ion is most probably m/e 204. The small peak at m/e 202 may indicate the presence of another component. The following fragment ions may be derived from m/e 204:

m/e 204 $[M]^{+\cdot}$	⟶ m/e 189	-15	($CH_3\cdot$)
	⟶ m/e 176	-28	($CH_2{=}CH_2$)
	⟶ m/e 175	-29	($CH_3CH_2\cdot$)
	⟶ m/e 163	-41	($CH_2{=}CHCH_2\cdot$)
	⟶ m/e 162	-42	($CH_2{=}CHCH_3$)
	⟶ m/e 161	-43	($C_3H_7\cdot$, $CH_3\cdot$ and $CH_2{=}CH_2$)
	⟶ m/e 148	-56	($CH_2{=}CHCH_2CH_3$, $CH_3CH{=}CHCH_3$)

The two most likely formulas that fit M+1 and M+2 data are $C_{15}H_{24}$ and $C_{14}H_{20}O$ (234). The formula $C_{15}H_{24}$ may be assigned for m/e 204 as the presence of oxygen was not indicated from peaks of lower mass.

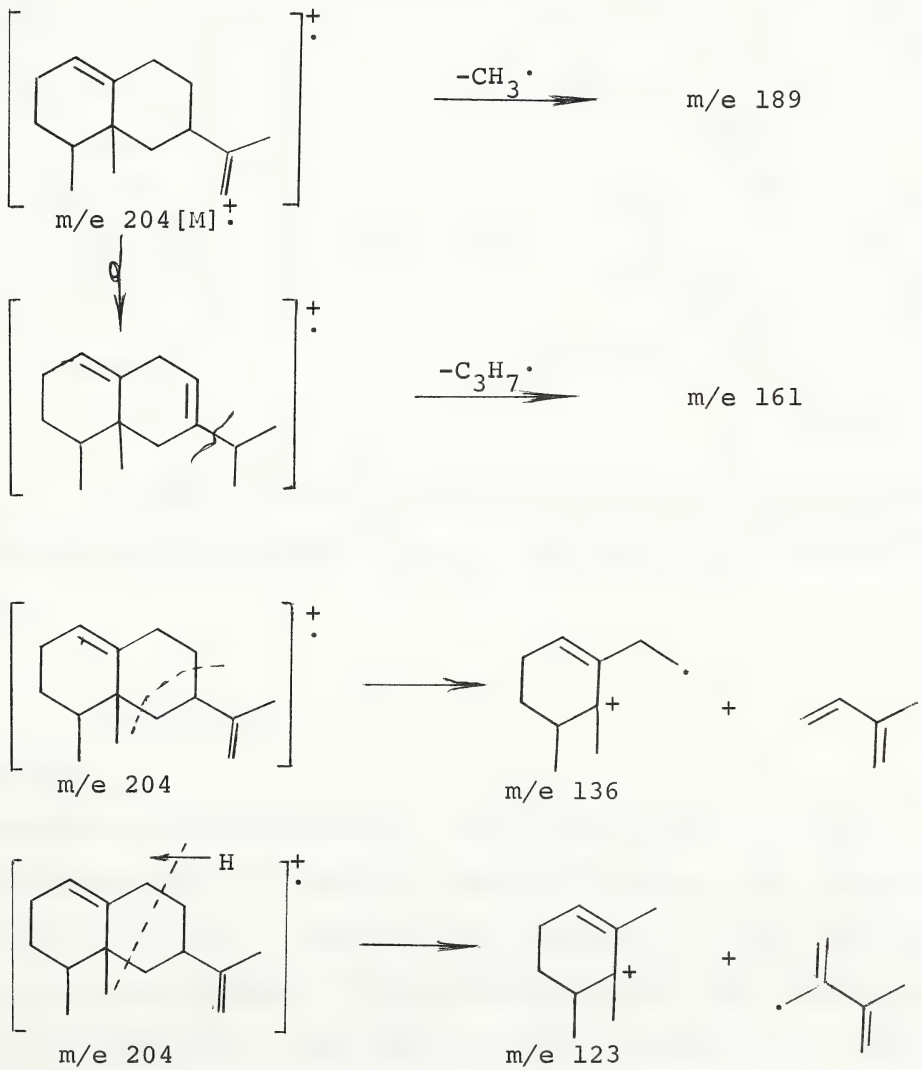
The intensity of the molecular ion suggested a cyclic structure. The intense m/e 55 peak with no complimentary M-55 ion suggested a cyclohexane ring system (218).

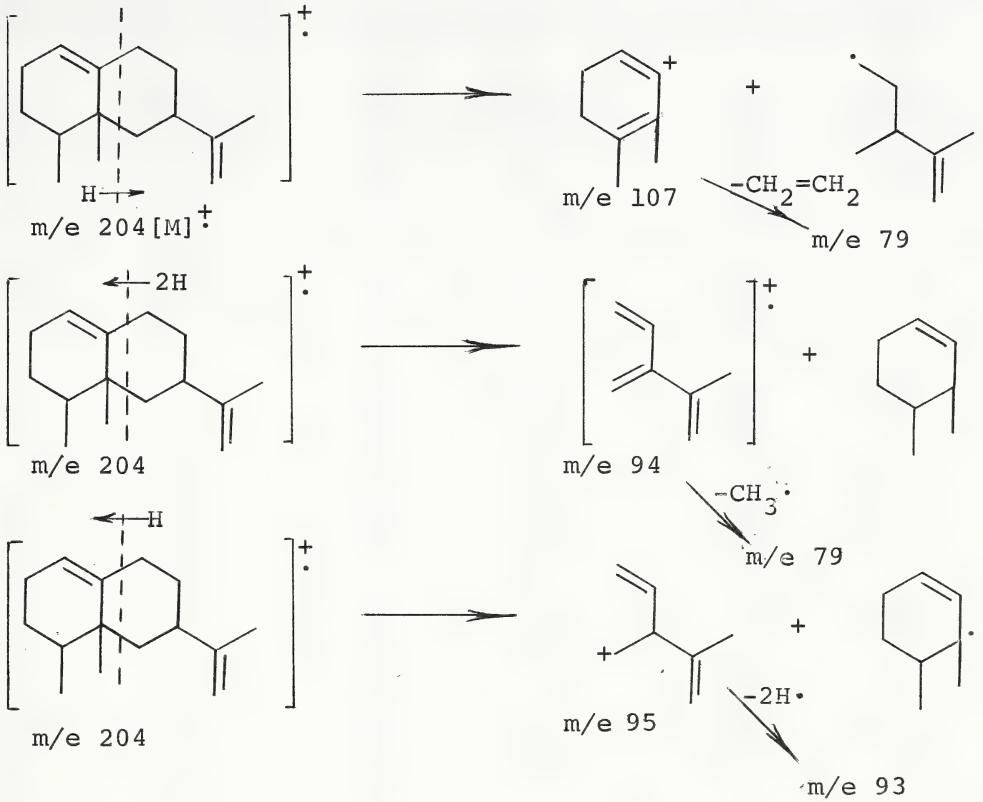
Comparison with the mass spectra of 24 sesquiterpene hydrocarbons (230) pointed to the similarity of the mass spectrum of this fraction with that of eremophilene, but they were not identical.

The intense peaks in the mass spectrum of eremophilene may be explained by a postulated fragmentation pathway in which the following considerations were observed. Migration

of the double bond, exocyclic to six membered ring, into the ring may occur on electron bombardment (235, 203). The rings may cleave at any position shown by dotted lines with or without hydrogen migration. The stability of the neutral fragment has control on the nature of the ions formed (236).

Eremophilene





It may be concluded that Fraction 18 contains a sesquiterpene hydrocarbon ($C_{15}H_{24}$) which may be of eremophilane type.

Fraction 19

Results

Gas-liquid chromatography: this fraction had a slightly broadened peak, a retention time of 19 mins., and represented 2.11% of the oil. The peak was augmented by both terpinyl acetate and pulegone. The collected liquid was colorless with slight odor. The quantity was too small to be tested

on the Se-30 column.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	1.0	49	0.6	73	12.9
13	0.6	50	3.9	74	1.0
14	2.9	51	8.4	75	1.6
15	5.9	52	3.9	76	0.3
16	0.3	53	24.8	77	15.5
17	1.3	54	10.6	78	3.5
19	1.9	55	70.6	79	24.2
26	5.9	56	20.6	80	6.1
27	51.0	57	41.3	81	34.2
29	51.9	58	6.8	82	13.9
30	1.6	59	16.5	83	25.2
31	11.0	60	2.3	84	8.7
37	0.6	61	1.9	85	10.3
38	2.6	62	0.3	86	3.9
39	41.9	63	2.6	87	2.2
40	8.1	64	1.0	88	0.6
41	95.2	65	8.7	89	1.0
42	23.2	66	5.2	90	0.3
43	100.0	67	43.5	91	21.9
44	8.4	68	13.2	92	6.1
45	16.1	69	29.4	93	21.6
46	1.3	70	19.0	94	7.1
47	1.3	71	30.6	95	21.3
48	0.6	72	5.5	96	8.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
97	11.9	123	6.1	150	0.3
98	5.5	124	3.5	151	1.6
99	8.4	125	3.9	152	1.0
100	2.3	126	1.3	153	1.0
101	12.9	127	4.2	155	1.0
102	2.6	128	2.6	156	0.3
103	3.5	129	4.2	157	1.0
104	1.6	130	1.6	158	0.3
105	18.4	131	3.5	159	5.5
106	6.5	132	1.9	160	1.9
107	11.6	133	6.8	161	14.2
108	3.5	134	2.9	162	3.2
109	8.1	135	3.5	163	0.6
110	7.1	136	1.9	164	0.3
111	8.1	137	1.9	165	1.0
112	4.8	138	2.3	166	0.3
113	5.2	139	1.3	167	0.3
114	1.3	140	1.0	168	0.6
115	2.6	141	1.9	169	0.6
116	1.9	142	1.3	170	0.3
117	6.1	143	2.9	171	0.3
118	1.9	144	1.0	173	0.6
119	12.3	145	5.9	174	0.3
120	4.8	146	1.3	175	1.0
121	6.1	147	2.6	176	1.0
122	2.6	148	2.3	177	1.6

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
178	0.5	187	1.6	195	0.3
179	1.0	188	0.3	196	0.3
180	0.3	189	3.5	202	1.9
181	0.5	190	1.0	203	0.3
182	0.3	191	0.3	204	6.8
183	0.3	193	0.6	205	1.9
184	0.3	194	0.6	206	0.3
				207	1.3

M(204) = 6.8

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
37.0	$121^+ \longrightarrow 67^+ + 54$
56.7	$105^+ \longrightarrow 77^+ + 28$
59.5	$105^+ \longrightarrow 79^+ + 26$
58.3	$107^+ \longrightarrow 79^+ + 28$
47.4	$95^+ \longrightarrow 67^+ + 28$
66.3	$93^+ \longrightarrow 79^+ + 14$
89.0	$93^+ \longrightarrow 91^+ + 2$
46.4	$91^+ \longrightarrow 65^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$

Ultraviolet data: a broad shoulder from λ350 - 220 mμ.

Infrared data:

3400 (m, broad), 3080 (w, sharp), 2930 (s), 2850 (s), 1720 (m), 1650 (m, sharp), 1445 (m), 1385 (sh), 1365 (m), 875 (m, sharp) cm^{-1}.

An infrared spectrum of an authentic sample of terpinyl acetate, $C_{12}H_{20}O_2$, was recorded: 3065(sh), 2960 (m, sharp), 2900 (s), 1730 (m), 1640 (m), 1430 (m), 1370 (m), 1250 (m), 1210 (w), 1145 (w), 1125 (w), 1100 (w), 1040 (w), 1005 (w), 900 (w), 875 (m,sharp), 785 (m) cm^{-1}.

An infrared spectrum of an authentic sample of pulegone, $C_{10}H_{16}O$, was recorded: 3340 (w, broad), 2930 (s), 2860 (m), 1780 (s), 1605 (s), 1440 (m), 1365 (m), 1330 (w), 1280 (s, sharp), 1200 (s, sharp), 1120 (m), 1085 (w), 1015 (w), 925 (w), 860 (w) cm^{-1}.

Discussion and conclusions

The retention data suggested pulegone or terpinyl acetate, but both were ruled out on comparison of the infrared and mass spectra (211).

At the high mass end, beside the distinct peaks at m/e 204, 189 and 161, there were small peaks at m/e 202, 205 and 207, indicating that m/e 204 may be the molecular ion of one of the components of this fraction or a fragment ion.

Peaks at m/e 31, 45, 59, 73 and 101 indicated the presence of oxygen. The number of carbon atoms could be traced up to C_{12}.

The infrared spectrum confirmed the aliphatic nature of the major component of this fraction by the strong saturated C-H stretching and bending absorption at 2930 and 1445 cm^{-1}. Aromaticity was excluded by the absence of strong absorption in the aromatic region 650 - 900 cm^{-1}, due to C-H out-of-plane bending, and C=C stretching of aromatic rings

in the region 1500 - 1600 cm^{-1}.

The C-H and C=C stretching bands at 3080 and near 1650 cm^{-1} indicated the olefinic nature which was confirmed by the C-H out-of-plane bending at 875 cm^{-1}.

The moderate absorption at 1720 cm^{-1} suggested α,β-unsaturated ester, and the moderate broad absorption at 3400 cm^{-1} suggested alcohol. However, the region 950 - 1200 cm^{-1} was poorly resolved and no information about the C-O stretching of alcohols or esters could be derived.

The ultraviolet data indicated diunsaturated nonconjugated chromophore (237).

It may be concluded that Fraction 19 contains more than one component. The major component is aliphatic oxygenated hydrocarbon with more than 12 carbon atoms.

Fraction 20

Results

Gas-liquid chromatography: this fraction had a small blunt peak, a retention time of 19.9 mins., and represented 1.56% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	26	4.5	39	47.5
13	0.5	27	47.5	40	9.0
14	1.5	29	56.5	41	97.5
15	5.5	31	3.5	42	17.5
17	0.5	38	3.0	43	100.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
44	6.0	73	2.5	101	2.0
45	4.5	74	1.0	102	0.5
46	0.5	75	1.5	103	1.5
47	0.5	77	14.0	104	1.0
50	2.5	78	3.0	105	14.0
51	7.0	79	23.5	106	4.5
52	4.5	80	7.5	107	9.0
53	29.0	81	49.0	108	6.5
54	10.0	82	16.0	109	44.5
55	68.0	83	28.5	110	10.5
56	18.0	84	8.0	111	7.0
57	37.5	85	6.0	112	2.5
58	4.0	86	3.0	113	1.5
59	2.0	87	1.0	114	0.5
60	1.0	89	1.0	115	2.0
62	0.5	90	0.5	116	1.0
63	3.0	91	15.5	117	3.5
64	1.0	92	4.0	118	1.0
65	9.5	93	20.0	119	6.5
66	6.0	94	15.5	120	2.0
67	24.5	95	10.0	121	5.5
68	19.5	96	5.0	122	1.5
69	29.0	97	9.0	123	3.0
70	29.0	98	6.5	124	6.0
71	13.0	99	4.5	125	4.5
72	3.0	100	1.0	126	2.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
127	3.5	143	1.0	167	0.5
128	1.0	144	0.5	168	0.5
129	4.0	145	4.5	169	0.5
130	1.5	146	1.0	175	0.8
131	2.5	147	3.0	177	0.5
132	1.0	148	1.5	179	0.5
133	4.0	150	0.5	187	0.5
134	2.0	151	1.0	189	3.0
135	3.0	152	0.5	190	0.5
136	3.0	153	0.5	193	0.5
137	3.0	159	2.0	194	0.5
138	1.0	161	6.5	202	0.5
139	0.5	162	2.0	204	4.0
140	1.0	163	1.0	205	1.5
141	1.0	165	1.0	206	0.5
142	0.5	166	0.5	207	0.5

M(204) = 4.0

Base peak: at m/e 43

Molecular formula: $C_{15}H_{24}$

Double bond equivalents: four

Metastable ions:

Diffuse peaks	Possible break-down reactions
60.5	$204^+ \longrightarrow 111^+ + 93$
37.1	$121^+ \longrightarrow 67^+ + 54$
24.4	$69^+ \longrightarrow 41^+ + 28$
66.3	$93^+ \longrightarrow 79^+ + 14$
89.0	$93^+ \longrightarrow 91^+ + 2$
75.0	$79^+ \longrightarrow 77^+ + 2$
39.1	$83^+ \longrightarrow 57^+ + 26$

Discussion and conclusions

The molecular ion of the component of this fraction most probably is m/e 204. The number of carbon atoms could be traced up to C_{12}. There was no indication of the presence of oxygen. The molecular formula, $C_{15}H_{24}$, may be assigned tentatively to m/e 204.

Comparison with reference sesquiterpene hydrocarbons (230) gave no clue about the structure.

Fraction 21

Results

Gas-liquid chromatography: this fraction had a small broadened peak, a retention time of 20.7 mins., and represented 2.04% of the oil.

Mass spectral data: two different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	14	1.2	16	0.4
13	0.4	15	3.2	17	0.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
19	0.4	59	2.8	87	0.4
26	3.6	62	0.4	88	0.4
27	38.0	63	3.6	89	2.0
29	37.2	64	1.6	90	1.2
30	0.8	65	15.6	91	58.4
31	3.6	66	5.6	92	16.0
37	0.8	67	31.6	93	51.6
38	1.6	68	10.0	94	15.6
39	38.0	69	24.4	95	12.8
40	7.2	70	5.6	96	3.6
41	100.0	71	6.4	97	3.2
42	10.0	72	1.6	98	1.2
43	56.0	73	1.2	99	3.2
44	2.8	74	0.8	100	1.2
45	4.4	75	2.8	101	4.0
46	0.4	76	0.4	102	1.2
47	0.8	77	38.8	103	6.0
50	2.0	78	9.2	104	4.4
51	8.4	79	51.2	105	55.2
52	5.2	80	8.0	106	12.4
53	31.2	81	43.2	107	16.8
54	6.0	82	11.2	108	4.4
55	50.0	83	20.8	109	6.4
56	7.6	84	3.2	110	3.2
57	15.6	85	5.6	111	3.6
58	2.4	86	0.8	112	1.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
113	1.2	137	1.2	163	2.0
114	0.8	138	0.8	164	0.4
115	7.6	139	0.4	165	0.4
116	3.6	140	0.4	167	0.4
117	11.6	141	2.0	169	0.4
118	4.4	142	1.2	173	0.4
119	43.6	143	1.6	175	1.6
120	14.0	144	0.8	176	4.4
121	10.8	145	6.0	177	1.2
122	2.4	146	2.4	179	0.4
123	1.2	147	6.0	181	0.4
124	1.2	148	6.8	187	0.4
125	2.0	149	0.4	189	6.4
126	0.8	150	3.2	190	1.2
127	3.2	151	1.2	191	0.4
128	5.2	152	1.2	193	0.4
129	13.6	153	0.8	194	0.4
130	3.2	154	0.4	195	0.4
131	8.0	155	0.4	202	0.8
132	3.2	157	0.8	203	0.4
133	28.0	159	2.8	204	24.4
134	19.6	160	3.2	205	4.4
135	12.4	161	96.0	206	0.6
136	5.6	162	15.6	207	0.4

M(204) = 24.4

M+1(205) = 4.4

M+2(206) = 0.6

Isotope abundances:

m/e	% of M
204(M)	100.
205(M+1)	18.
206(M+2)	2.46

Base peak: at m/e 41

Molecular formula: $C_{15}H_{24}$

Double bond equivalents: four

Metastable ions:

Diffuse peaks	Possible break-down reactions
127.0	$204^+ \longrightarrow 161^+ + 43$
175.0	$204^+ \longrightarrow 189^+ + 15$
88.0	$204^+ \longrightarrow 134^+ + 70$
105.5	$134^+ \longrightarrow 119^+ + 15$
109.5	$161^+ \longrightarrow 133^+ + 28$
63.7	$136^+ \longrightarrow 93^+ + 43$
89.0	$93^+ \longrightarrow 91^+ + 2$
71.5	$121^+ \longrightarrow 93^+ + 28$
37.1	$121^+ \longrightarrow 67^+ + 54$
69.6	$119^+ \longrightarrow 91^+ + 28$
46.5	$91^+ \longrightarrow 65^+ + 26$
62.2	$111^+ \longrightarrow 83^+ + 28$
58.3	$107^+ \longrightarrow 79^+ + 28$
59.5	$105^+ \longrightarrow 79^+ + 26$
66.4	$93^+ \longrightarrow 79^+ + 14$
39.2	$83^+ \longrightarrow 57^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$

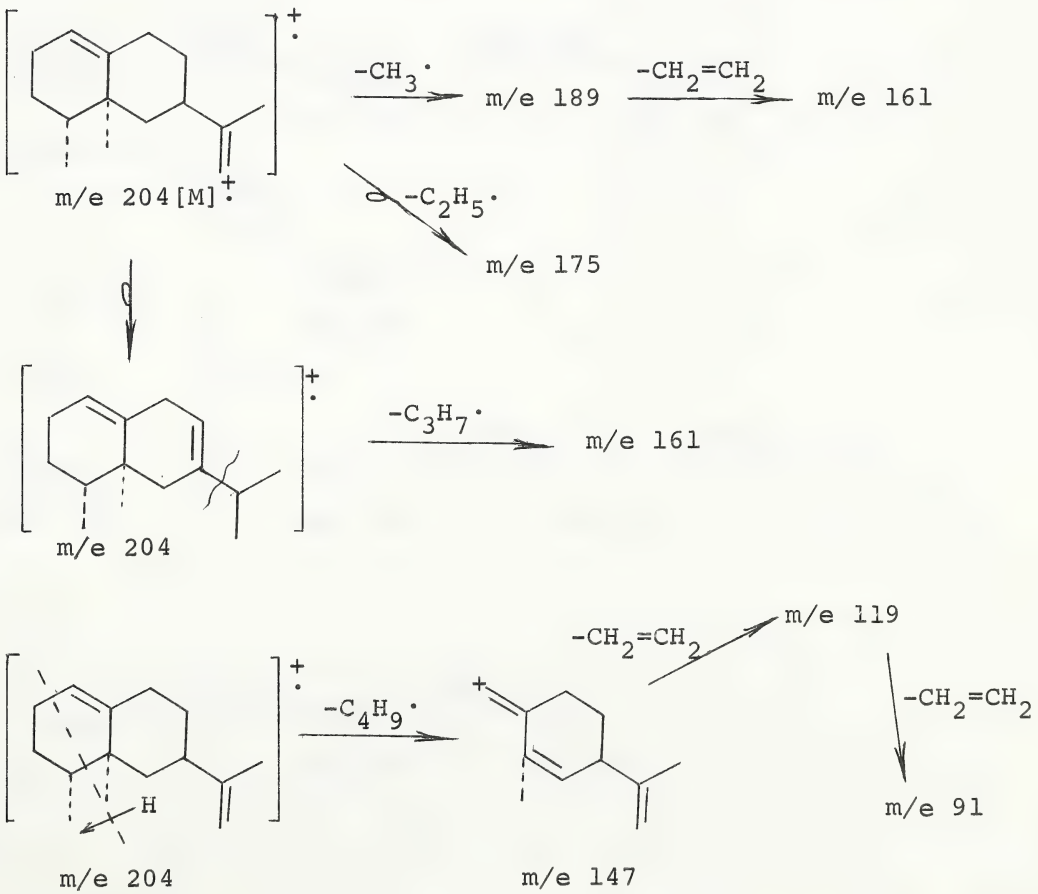
Discussion and conclusions

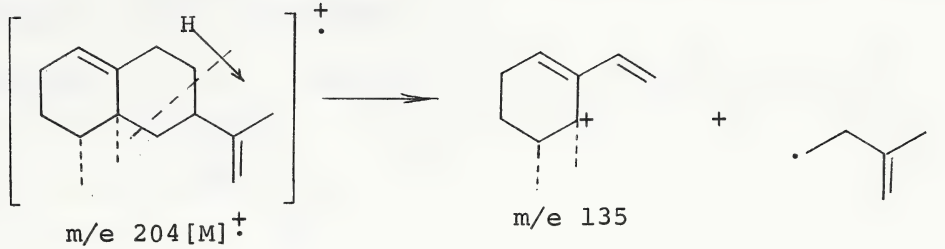
The isotopic abundances indicated the formula $C_{15}H_{24}$ for the intense molecular ion (m/e 204). The general appearance of the mass spectrum also suggested sesquiterpene.

Comparison with reference sesquiterpene hydrocarbons (230) indicated that the mass spectrum of this fraction was similar but not identical with that of valencene.

The following postulated fragmentation pathway may account for the intense peaks in the mass spectrum of valencene:

Valencene





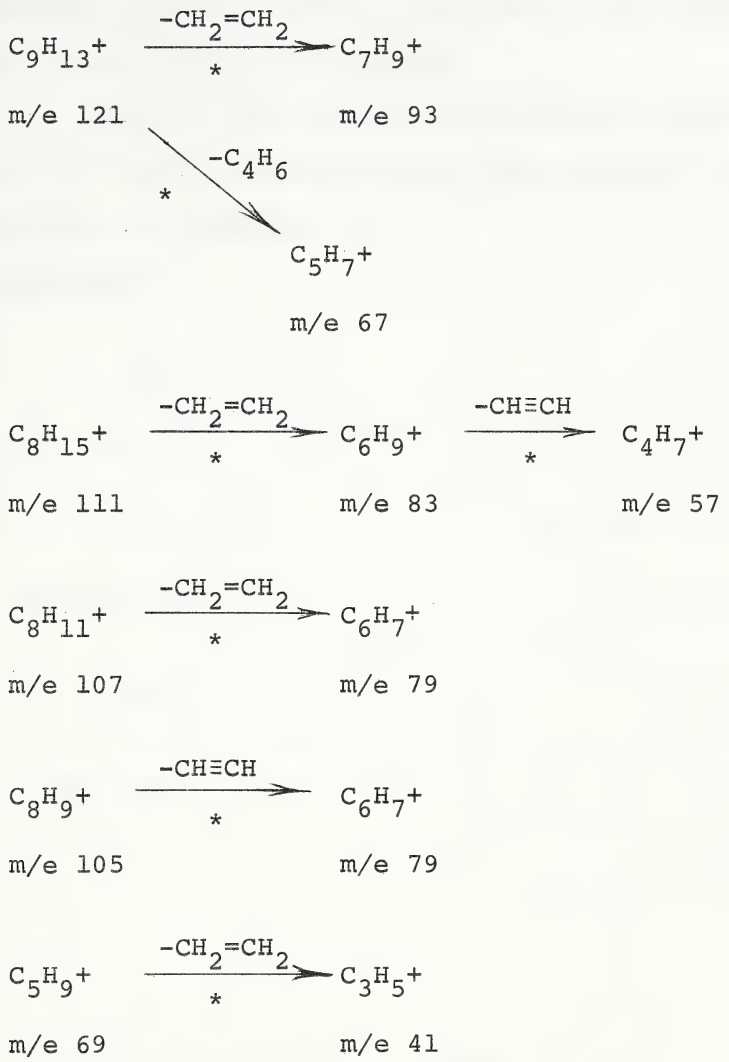
The following transitions in the fragmentation of component 21 were substantiated by suitable metastable ions:

$[C_{15}H_{24}]^{+\cdot}$ m/e 204[M]$^{+\cdot}$ $\xrightarrow[*]{-C_3H_7\cdot}$ $C_{12}H_{17}^+$ m/e 161 $\xrightarrow[*]{-CH_2=CH_2}$ $C_{10}H_{13}^+$ m/e 133

m/e 204[M]$^{+\cdot}$ $\xrightarrow[*]{-CH_3\cdot}$ $C_{14}H_{21}^+$ m/e 189

m/e 204[M]$^{+\cdot}$ $\xrightarrow[*]{-C_5H_{10}}$ $[C_{10}H_{14}]^{+\cdot}$ m/e 134 $\xrightarrow[*]{-CH_3\cdot}$ $C_9H_{11}^+$ m/e 119 $\xrightarrow[*]{-CH_2=CH_2}$ $C_7H_7^+$ m/e 91 $\xrightarrow[*]{-CH\equiv CH}$ $C_5H_5^+$ m/e 65

$[C_{10}H_{16}]^{+\cdot}$ m/e 136 $\xrightarrow[*]{-C_3H_7\cdot}$ $C_7H_9^+$ m/e 93 $\xrightarrow[*]{-2H\cdot}$ $C_7H_7^+$ m/e 91



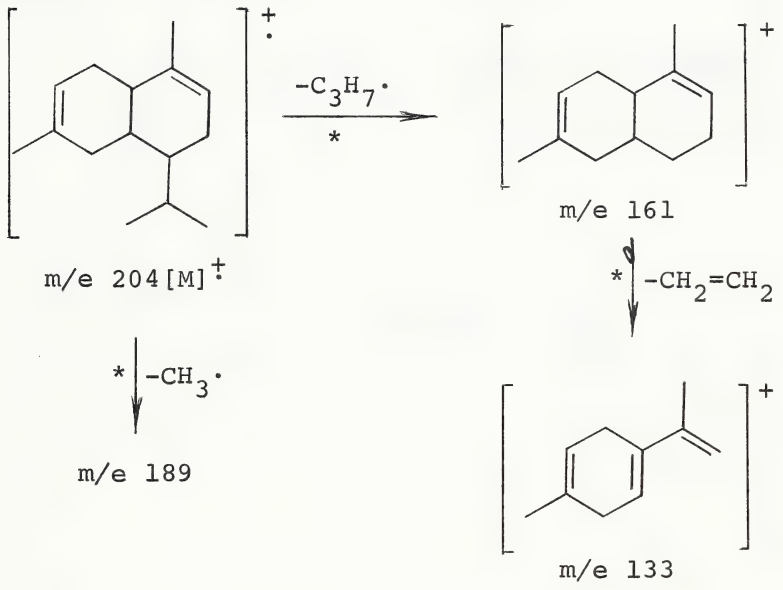
These transitions are almost identical to those of Fraction 31 which is most probably δ-cadinene (discussed later). The mass spectrum of this component is also closely related to that of Fraction 31.

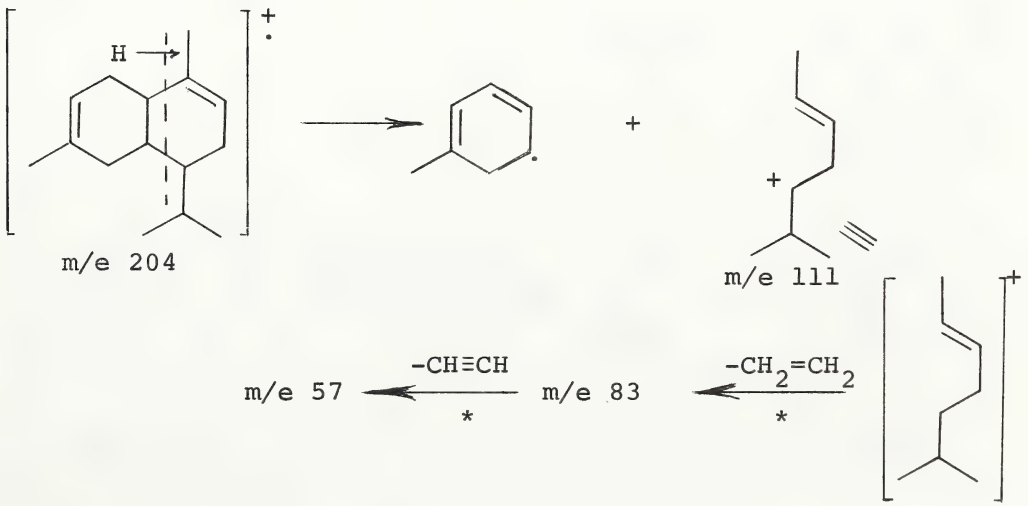
Cadinene is a bicyclic sesquiterpene widely distributed in plants (238). There are nine possible isomers of

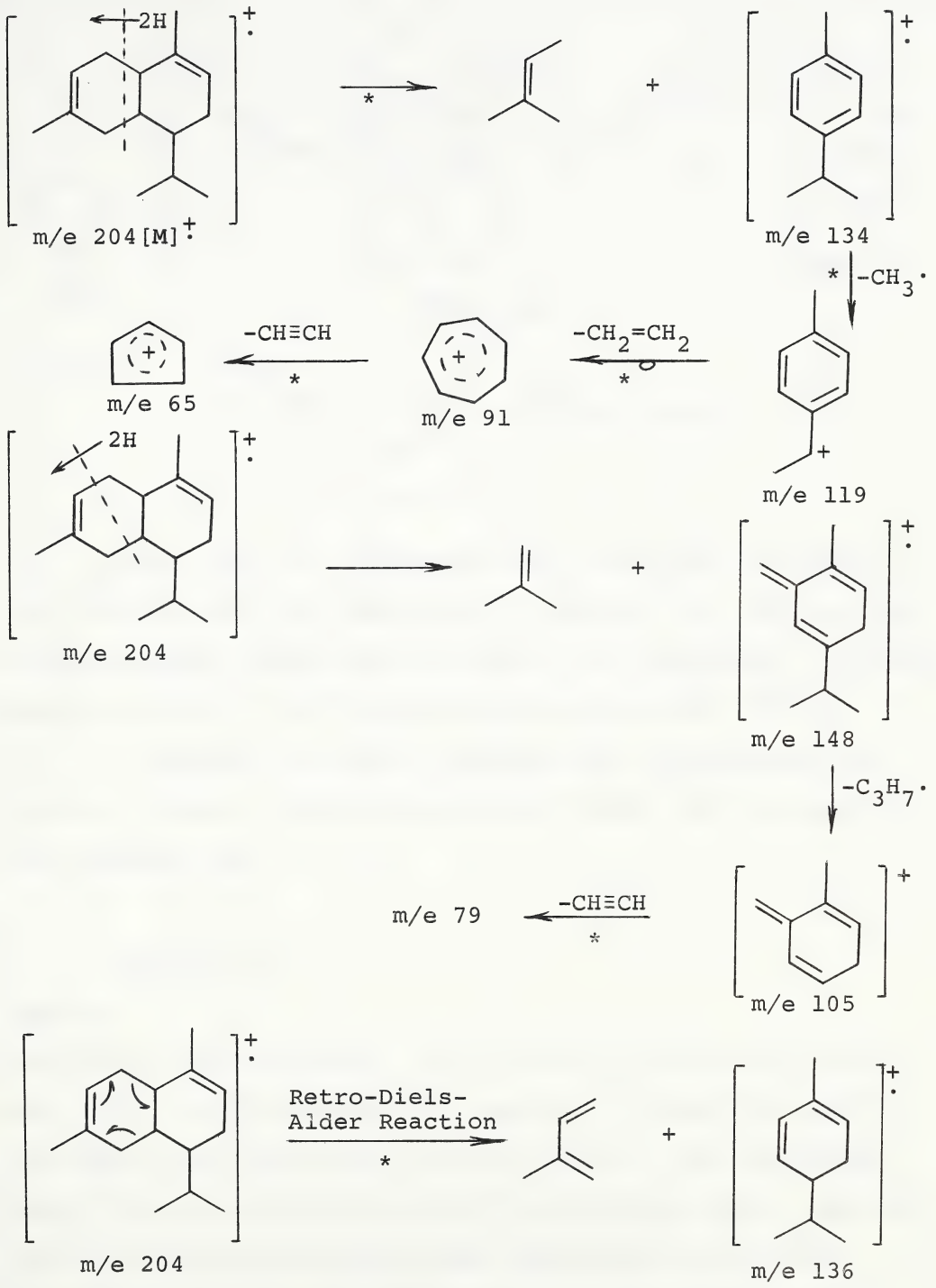
cadinene ($C_{15}H_{24}$) (239). Six isomers are known (240). The principle isomer is β-cadinene.

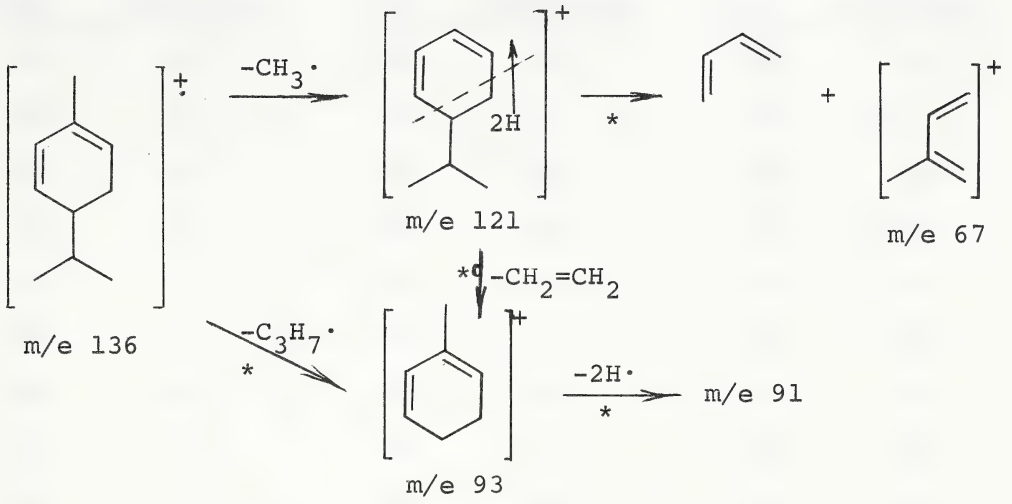
However the following postulated fragmentation pathway for β-cadinene may explain the prominent peaks in the mass spectrum of component 21:

β-cadinene






2H
m/e 204 [M]+·
*
+
m/e 134
* -CH3·
m/e 119
-CH≡CH
*
m/e 65
m/e 91
-CH2=CH2
*
2H
m/e 204
+
m/e 148
-C3H7·
m/e 79
-CH≡CH
*
m/e 105
Retro-Diels-
Alder Reaction
*
+
m/e 204
m/e 136



In the same way α-cadinene may account for the prominent peaks in the mass spectrum of component 21. However none of the cadinene isomers was available for comparison. The mass spectra of only δ- and γ-cadinene were published (230).

In summary, it may be concluded that Fraction 21 may contain a bicyclic sesquiterpene hydrocarbon, most probably of the cadinane type.

Fraction 31

Results

Gas-liquid chromatography: this fraction had a broad large peak which was overlapping with peak 30, a retention time of 34.6 mins., and represented 8.0% of the oil. The collected viscid liquid, which had a slight disagreeable smell, was purified on Se-30 column before mass spectrometric analysis.

Mass spectral data: three different scans were run on MS12, and one on MS9.

Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.6	51	3.8	78	5.2
13	0.2	52	2.6	79	29.6
14	0.8	53	15.6	80	4.8
15	2.4	54	2.8	81	33.6
16	0.2	55	31.2	82	3.6
19	0.2	56	4.2	83	3.6
20	0.1	57	7.2	84	1.8
25	0.6	58	11.8	85	5.2
26	2.2	59	2.4	86	0.4
27	21.0	60	0.2	87	0.4
29	18.6	62	0.2	88	0.4
30	0.4	64	0.6	89	1.0
31	1.8	65	7.6	90	0.6
37	0.4	66	3.0	91	25.2
38	0.6	67	15.8	92	6.6
39	17.0	68	3.6	93	24.8
40	4.0	69	17.6	94	9.4
41	55.0	70	3.2	95	23.8
42	5.0	71	15.6	96	2.8
43	80.0	72	1.0	97	2.4
44	2.6	73	0.8	98	1.4
45	4.0	74	0.4	99	1.0
46	0.2	75	1.0	100	0.6
47	0.2	76	0.4	101	0.8
50	1.0	77	21.4	102	0.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
103	2.8	129	2.2	157	0.6
104	2.0	130	1.2	158	0.4
105	34.6	131	4.2	159	2.6
106	6.0	132	1.6	160	2.6
107	8.6	133	8.8	161	100.0
108	3.8	134	20.0	162	18.0
109	7.6	135	5.2	163	2.4
110	1.4	136	1.6	164	2.0
111	1.8	137	2.6	165	0.4
112	0.4	139	0.2	166	0.6
113	0.6	141	0.8	167	0.2
114	0.4	142	0.6	168	0.2
115	3.2	143	1.4	169	0.3
116	1.6	144	0.6	173	0.4
117	4.2	145	4.8	174	0.2
118	3.4	146	2.2	175	1.2
119	21.0	147	4.8	176	1.2
120	5.8	148	1.6	177	0.4
121	18.0	149	1.2	178	0.2
122	2.8	150	0.6	179	0.4
123	2.2	151	0.8	181	0.4
124	0.4	152	0.6	187	0.6
125	1.4	153	0.8	189	11.6
126	0.6	154	0.4	190	2.0
127	2.6	155	0.4	191	0.2
128	2.2	156	0.1	193	0.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
194	0.2	202	0.6	205	5.6
195	0.2	203	0.2	206	0.6
196	0.2	204	30.0	207	0.4

M(204) = 30.0

M+1(205) = 5.6

M+2(206) = 0.6

Isotope abundances:

m/e	% of M
204(M)	100.
205(M+1)	18.7
206(M+2)	2.0

Base peak: at m/e 161

Molecular formula: $C_{15}H_{24}$

Double bond equivalents: four

Metastable ions:

Diffuse peaks	Possible break-down reactions
175.5	$204^+ \longrightarrow 189^+ + 15$
127.	$204^+ \longrightarrow 161^+ + 43$
88.0	$204^+ \longrightarrow 134^+ + 70$
105.5	$134^+ \longrightarrow 119^+ + 15$
109.5	$161^+ \longrightarrow 133^+ + 28$
63.7	$136^+ \longrightarrow 93^+ + 43$
89.0	$93^+ \longrightarrow 91^+ + 2$
71.5	$121^+ \longrightarrow 93^+ + 28$
37.1	$121^+ \longrightarrow 67^+ + 54$
69.6	$119^+ \longrightarrow 91^+ + 28$
66.4	$93^+ \longrightarrow 79^+ + 14$
38.1	$85^+ \longrightarrow 57^+ + 28$
24.4	$69^+ \longrightarrow 41^+ + 28$

Data from MS9:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.1	50	0.2	80	0.7
14	0.7	51	0.9	81	7.5
15	0.4	52	0.4	82	0.7
16	2.0	53	2.4	83	0.3
17	2.3	54	0.3	84	0.1
18	100.0	55	4.4	85	0.1
19	0.4	56	0.5	89	0.2
20	0.2	57	0.5	90	0.1
26	0.2	58	1.2	91	6.6
27	2.8	59	0.2	92	1.9
28	20.7	63	0.2	93	6.1
29	2.5	64	0.1	94	2.1
30	0.1	65	1.6	95	3.7
31	5.1	66	0.4	96	0.4
32	4.1	67	2.7	97	0.2
33	0.1	68	0.4	98	0.1
34	0.2	69	2.7	102	0.1
39	3.1	70	0.3	103	0.7
40	0.5	71	1.4	104	0.4
41	8.8	72	0.1	105	12.5
42	0.5	75	0.1	106	2.1
43	7.8	76	0.1	107	2.1
44	1.3	77	4.4	108	0.5
45	0.4	78	1.0	109	1.0
47	0.1	79	5.4	110	0.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
111	0.1	133	3.1	158	0.1
113	0.1	134	4.1	159	0.9
115	1.1	135	1.0	160	0.9
116	0.5	136	0.3	161	20.3
117	1.4	137	0.2	162	4.1
118	0.8	141	0.2	163	0.4
119	6.8	142	0.1	164	0.2
120	1.7	143	0.2	175	0.3
121	3.4	144	0.2	176	0.3
122	0.4	145	1.1	177	0.1
123	0.7	146	0.4	179	0.1
124	0.1	147	1.1	187	0.1
125	0.1	148	0.7	189	2.7
127	0.2	149	0.5	190	0.3
128	0.6	150	0.2	202	0.1
129	0.7	151	0.1	203	0.1
130	0.3	152	0.1	204	8.1
131	1.0	153	0.1	205	1.2
132	0.4	157	0.2	206	0.1
				207	0.1

An accurate mass determination of the molecular ion (m/e 204) was made:

measured 204.1874; calculated for $C_{15}H_{24}$:204.1878.

Discussion and conclusions

The isotopic abundances (MS12) and the precise mass measurements made with MS9 indicated the formula $C_{15}H_{24}$ for

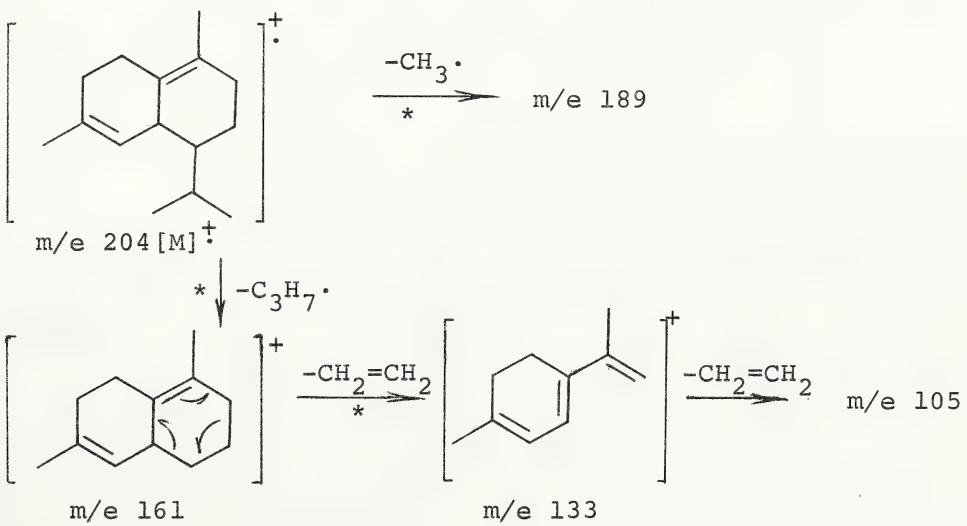
the molecular ion (m/e 204). The m/e 31, 45 and 59 peaks, which indicated the presence of oxygen, are most probably related to Fraction 30. It seems that complete separation of the two overlapping Fractions 30 and 31 was not accomplished. The presence of oxygen was obvious in Fraction 30 which is discussed later.

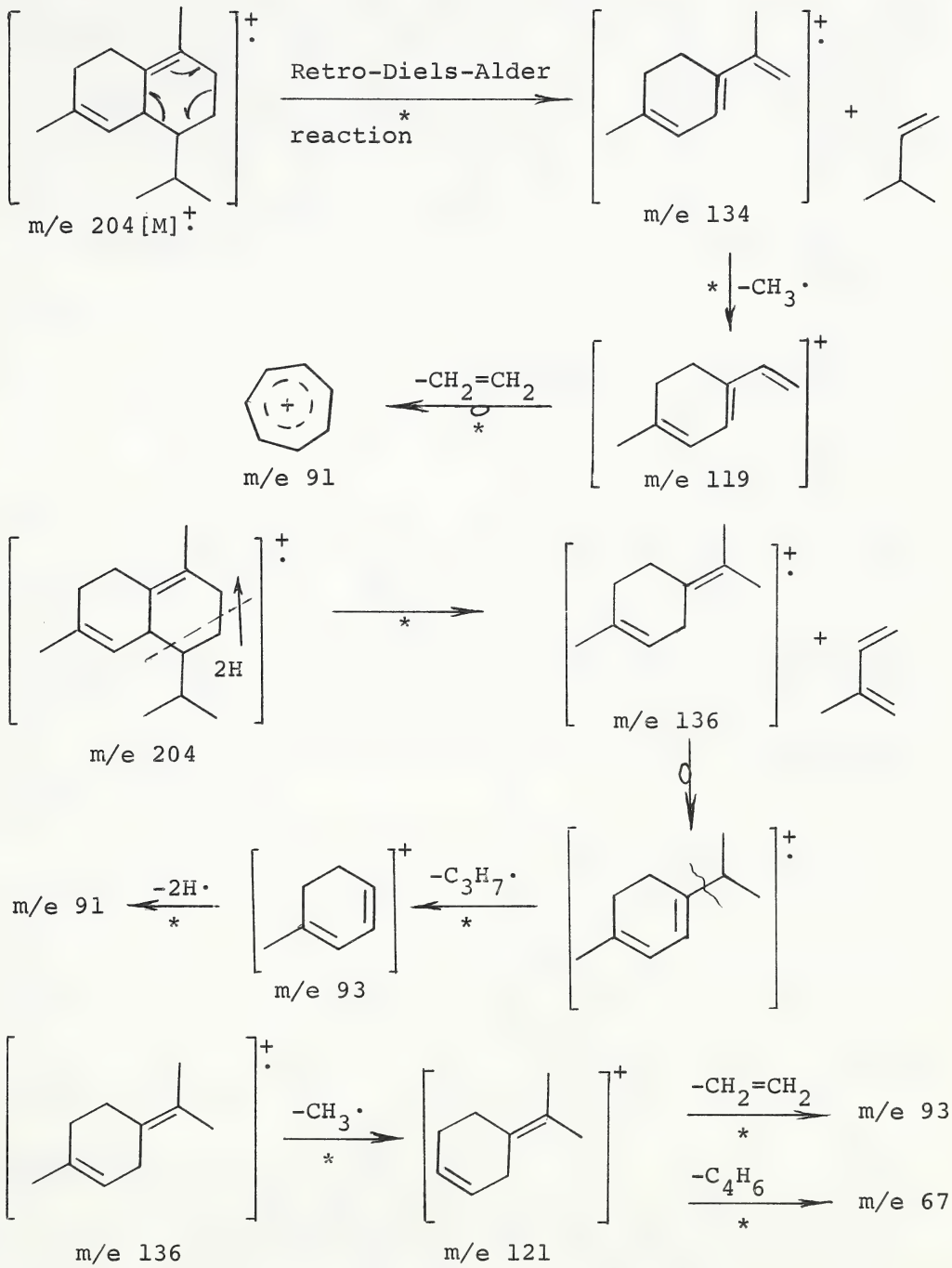
The ultraviolet and infrared spectra were run on a sample containing both fractions. However no aromaticity was indicated.

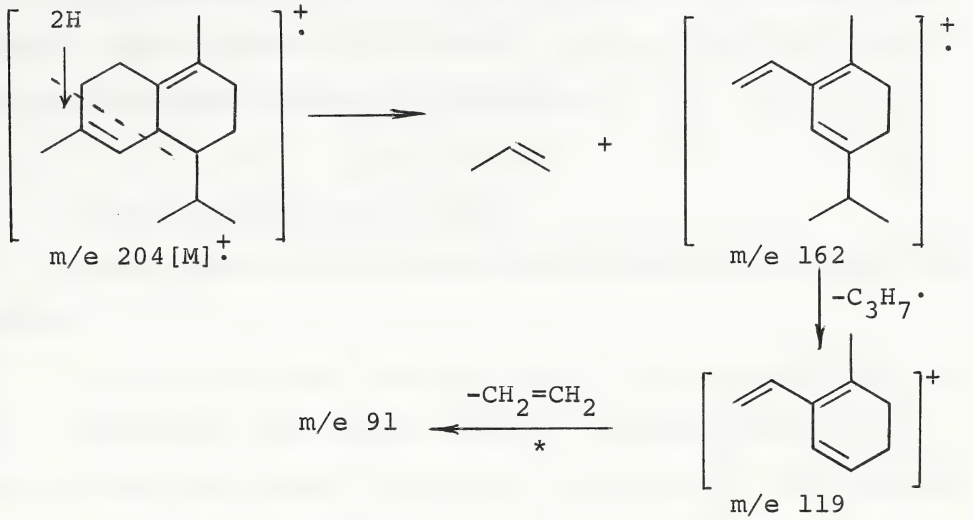
On comparison with reference sesquiterpene hydrocarbons, the mass spectrum (MS12) of this fraction was very similar to those of δ-cadinene (227, 230).

The following postulated fragmentation pathway for δ-cadinene may account for the intense peaks in the mass spectra of Fraction 31. Some of the transitions are substantiated by metastable ions.

δ-Cadinene



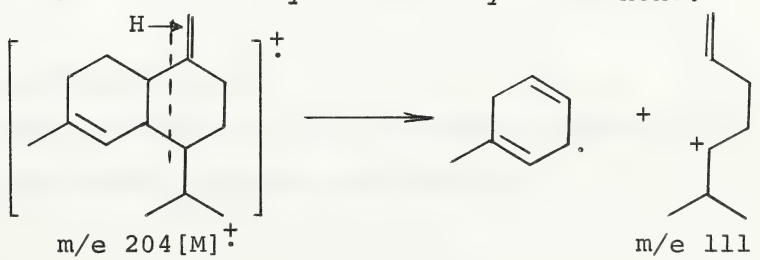

Retro-Diels-Alder
reaction
*
m/e 204 [M]+·
m/e 134
-CH3·
-CH2=CH2
m/e 91
m/e 119
2H
m/e 204
m/e 136
-2H·
m/e 91
-C3H7·
m/e 93
-CH3·
m/e 136
m/e 121
-CH2=CH2
m/e 93
-C4H6
m/e 67



The mass spectra of δ- and γ-cadinene (230) were very similar below m/e 105. The intense peaks at m/e 134 and 119 in the spectrum of δ-cadinene were very small or missing in γ-cadinene, while the distinct peak at m/e 111 in the spectrum of γ-cadinene was missing in δ-cadinene. These differences may be rationalized.

The odd-electron ion m/e 134 may be derived from the molecular ion of δ-cadinene by a retro-Diels-Alder reaction which can not be afforded by γ-cadinene. The loss of a methyl radical from m/e 134 yields m/e 119. The m/e 111 ion may be derived from the molecular ion of γ-cadinene by the following cleavage which can not be easily achieved by δ-cadinene.

γ-Cadinene



In conclusion, Fraction 31 contains as its major component a sesquiterpene hydrocarbon, $C_{15}H_{24}$, which may be of the cadinane type, probably δ-cadinene.

Fractions pre 22, 23 and 30

These fractions had some common features in their mass spectra.

At the high mass end there were distinct peaks at m/e 220. There were also peaks probably corresponding to the loss of water and methyl radicals from m/e 220. The loss of water indicated the presence of a hydroxyl group (241). Intense peaks corresponding to m/e 220-H_2O-43 were also present.

The general appearance of the mass spectra, with clusters of peaks 14 mass units apart, indicated the alkyl hydrocarbon nature of the components of these fractions. The number of carbon atoms could be traced up to C_{12}. The m/e 43 peak was the base peak or the third most intense peak.

The presence of oxygen could be detected from the distinct peaks at m/e 31, 45, 59 and 115.

The mass spectra of Fractions pre 22 and 23 looked more similar to each other and distinctly different from that of 30.

Fraction pre 22 and 23

These two fractions will be discussed together because of the similarity of their mass spectra.

Fraction pre 22

Results

Gas-liquid chromatography: this fraction had a very small peak which could not be measured, and a retention time of 21.6 mins.

Mass spectral data: two different scans were run on MS12 which were not completely identical.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	1.0	45	6.5	65	10.0
13	1.0	46	0.5	66	4.5
14	3.5	47	0.5	67	29.5
15	5.0	48	0.5	68	13.0
19	1.0	49	0.5	69	45.5
20	1.0	50	3.0	70	10.0
26	5.5	51	8.5	71	18.0
27	39.0	52	2.5	72	3.5
29	40.5	53	20.5	73	3.5
30	0.5	54	10.5	74	0.5
31	6.0	55	54.5	75	2.0
37	1.0	56	15.0	76	0.5
38	3.0	57	34.0	77	15.5
39	45.0	58	5.5	78	3.0
40	8.5	59	3.5	79	23.5
41	92.5	60	0.5	80	4.0
42	13.5	62	0.5	81	30.0
43	91.0	63	4.5	82	16.5
44	5.0	64	1.0	83	16.5

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
84	5.5	110	7.0	136	1.0
85	12.5	111	8.0	137	2.5
86	3.0	112	5.5	138	2.5
87	1.5	113	3.0	139	1.5
88	1.0	114	2.0	140	2.0
89	1.0	115	10.0	141	7.5
90	0.5	116	3.5	142	4.5
91	19.5	117	6.0	143	8.5
92	4.5	118	2.0	144	9.0
93	24.5	119	10.5	145	8.0
94	8.0	120	4.0	146	2.0
95	16.5	121	5.5	147	3.5
96	5.5	122	2.5	148	1.5
97	9.0	123	6.0	150	2.5
98	4.0	124	2.5	151	2.5
99	10.5	125	6.5	152	1.5
100	2.0	126	3.5	153	1.5
101	4.5	127	8.5	155	2.6
102	1.5	128	18.5	156	1.5
103	3.5	129	13.0	157	5.0
104	2.0	130	3.5	158	3.5
105	17.0	131	12.5	159	100.0
106	3.5	132	3.0	160	15.0
107	14.0	133	4.5	161	5.5
108	13.0	134	3.0	162	2.0
109	12.0	135	5.5	163	2.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
164	1.0	179	1.0	195	0.5
165	1.5	181	1.0	196	0.5
166	1.5	182	0.5	200	1.0
167	1.0	183	1.0	201	0.5
168	0.5	184	1.0	202	10.0
169	0.5	185	1.0	203	2.5
171	1.0	187	1.5	204	3.5
173	1.5	189	1.5	205	2.5
174	2.5	191	1.0	206	1.0
175	1.5	192	0.5	207	1.5
177	1.5	193	0.5	220	1.5
178	1.0	194	0.5	221	0.5

Base peak: at m/e 159

Metastable ions:

Diffuse peaks	Possible break-down reactions
63.7	$136^+ \longrightarrow 93^+ + 43$
89.0	$93^+ \longrightarrow 91^+ + 2$
37.1	$121^+ \longrightarrow 67^+ + 54$
58.5	$107^+ \longrightarrow 79^+ + 28$
25.1	$67^+ \longrightarrow 41^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$

Fraction 23

Results

Gas-liquid chromatography: this fraction had a small blunt

peak, a retention time of 24.4 mins., and represented 1.82% of the essential oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.9	47	0.3	71	22.0
13	0.6	48	0.5	72	2.6
14	2.1	49	0.6	73	2.1
15	5.0	50	2.9	74	0.9
16	0.3	51	6.8	75	2.4
19	0.6	52	3.5	76	0.6
24	0.6	53	19.7	77	13.2
25	0.9	54	5.3	78	2.4
26	4.4	55	46.7	79	15.3
27	28.8	56	10.3	80	4.7
29	29.7	57	28.8	81	28.2
30	0.9	58	7.9	82	10.0
31	4.7	59	3.5	83	17.4
37	0.9	60	1.2	84	5.0
38	2.1	62	0.9	85	17.9
39	29.4	63	3.8	86	2.1
40	5.6	64	1.2	87	1.2
41	69.1	65	7.9	88	0.6
42	9.7	66	4.1	89	1.8
43	100.0	67	22.0	90	0.7
44	5.6	68	12.4	91	12.9
45	5.3	69	32.6	92	3.5
46	0.3	70	6.8	93	22.6

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
94	4.7	121	6.8	150	0.9
95	11.8	122	1.8	151	3.2
96	5.6	123	4.4	152	3.2
97	6.5	124	1.2	153	5.0
98	2.6	125	6.5	154	1.8
99	5.6	126	12.1	155	5.0
100	2.1	127	10.3	156	9.1
101	2.1	128	9.7	157	50.0
102	1.5	129	6.5	158	12.4
103	2.1	130	2.1	159	4.4
104	1.8	131	3.5	160	1.2
105	9.4	132	1.8	161	3.8
106	2.6	133	4.1	162	1.5
107	10.0	134	1.8	163	1.5
108	15.6	135	4.1	164	0.9
109	12.9	136	2.1	165	2.1
110	4.1	137	2.6	166	0.9
111	17.9	138	5.2	167	0.9
112	2.4	139	2.9	168	0.9
113	1.8	140	2.6	169	0.9
115	10.0	141	17.1	170	0.6
116	2.6	142	26.2	171	0.9
117	3.2	143	8.7	172	0.6
118	1.5	145	5.8	173	8.5
119	7.1	147	2.9	174	1.2
120	2.9	149	0.3	175	1.5

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
176	0.6	192	0.6	210	0.3
177	2.1	193	0.6	211	0.3
178	0.9	194	0.6	212	0.3
179	0.9	196	0.6	214	0.5
181	0.9	198	0.6	217	0.5
182	0.9	200	7.1	218	0.3
183	1.5	201	2.4	219	1.5
184	1.2	202	1.2	220	5.3
185	1.2	203	0.9	221	2.1
186	0.5	204	2.1	222	0.3
187	1.2	205	24.0	223	0.6
188	2.9	206	3.8	234	0.6
189	2.4	207	1.8	235	0.3
190	0.9	208	0.6	236	0.3
191	1.2	209	0.3	237	1.5
				238	0.3

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
129.5	$236^+ \longrightarrow 175^+ + 61$
129.0	$205^+ \longrightarrow 162^+ + 43$
78.9	$202^+ \longrightarrow 126^+ + 76$
79.6	$200^+ \longrightarrow 126^+ + 74$
80.0	$177^+ \longrightarrow 119^+ + 58$
64.0	$141^+ \longrightarrow 95^+ + 46$
129.4	$158^+ \longrightarrow 143^+ + 15$
139.0	$143^+ \longrightarrow 141^+ + 2$
140.0	$142^+ \longrightarrow 141^+ + 1$
25.1	$129^+ \longrightarrow 57^+ + 72$
93.5	$127^+ \longrightarrow 109^+ + 18$
92.5	$126^+ \longrightarrow 108^+ + 18$
56.7	$105^+ \longrightarrow 77^+ + 28$
36.5	$83^+ \longrightarrow 55^+ + 28$
39.1	$83^+ \longrightarrow 57^+ + 26$
63.0	$71^+ \longrightarrow 67^+ + 4$
24.4	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions

The mass spectra of Fractions pre 22 and 23 were almost identical at the low mass end up to m/e 93.

At the high mass end the distinct peaks immediately below m/e 220, which could not be derived from it, indicated that (m/e 220) is not the molecular ion.

The small peak at m/e 238 in the mass spectrum of Fraction 23 is probably due to the molecular ion. The

following fragment ions may be derived from m/e 238:

m/e 238	m/e 237	-1	(H·)
	m/e 221	-17	(OH·)
	m/e 220	-18	(H_2O)

The following fragment ions may be derived from m/e 220:

m/e 220	m/e 219	-1	(H·)
	m/e 205	-15	(CH_3·)
	m/e 202	-18	(H_2O)
	m/e 200	-20	(2H·, H_2O)

The loss of 18 mass units from m/e 238 and 220 revealed the presence of hydroxyl groups (225).

The intense peak at m/e 157 may be derived from m/e 200 by the loss of 43 mass units. This, together with the base peak at m/e 43, may suggest an isopropyl group (242).

The following fragment ions may be derived from m/e 220 in the mass spectrum of Fraction pre 22:

m/e 220	205	-15	(CH_3·)
	203	-17	(OH·)
	202	-18	(H_2O)

The fragment ion m/e 202 seems to lose 43 mass units to give rise to the base peak (m/e 159). This loss of 43 mass units and the intense m/e 43 also suggested an isopropyl group.

It may be concluded that Fractions pre 22 and 23 may contain sesquiterpene alcohols. The tentative formula $C_{15}H_{26}O_2$ may be given to the component of Fraction 23.

Fraction 30

Results

Gas-liquid chromatography: this fraction had a large peak which was overlapping with 31, a retention time of 33.9 mins., and represented 4.28 % of the oil.

Mass spectral data: three different scans were run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	50	1.4	70	6.0
13	0.2	51	6.2	71	11.7
14	1.2	52	3.8	72	1.4
15	5.0	53	23.3	73	1.4
16	0.2	54	6.9	74	0.5
19	0.2	55	38.3	75	1.6
25	0.7	56	6.0	76	0.5
26	2.6	57	10.0	77	26.7
27	25.0	58	5.7	78	7.6
29	24.5	59	2.1	79	30.5
30	0.7	60	0.2	80	8.6
31	2.6	61	7.1	81	19.8
39	29.8	62	0.5	82	8.8
40	6.2	63	3.1	83	11.4
41	78.6	64	1.2	84	2.9
42	9.3	65	12.1	85	5.2
43	100.0	66	4.5	86	0.7
44	4.8	67	26.0	87	0.2
45	3.5	68	4.5	89	1.2
46	0.1	69	23.3	90	0.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
91	40.7	117	13.8	144	2.1
92	9.3	118	4.3	145	14.0
93	26.0	119	23.8	146	7.6
94	6.4	120	7.4	147	9.3
95	11.2	121	8.8	148	2.9
96	2.4	122	2.6	149	3.8
97	4.0	123	3.3	150	1.4
98	3.3	124	1.0	151	0.7
99	1.6	125	2.1	152	0.5
100	0.5	126	0.7	153	0.5
101	1.0	127	2.4	154	0.2
102	1.0	128	4.5	155	0.7
103	4.3	129	5.2	156	0.5
104	4.0	130	2.9	157	1.4
105	32.9	131	23.8	158	1.2
106	9.5	132	5.7	159	21.4
107	15.5	133	10.7	160	5.0
108	4.5	134	6.9	161	9.8
109	6.2	135	6.0	162	7.4
110	1.4	136	1.6	163	1.9
111	1.4	137	1.6	164	0.5
112	0.5	139	0.2	165	0.2
113	0.5	140	0.5	166	0.2
114	0.5	141	2.4	167	0.2
115	7.1	142	1.4	169	0.2
116	3.3	143	4.5	170	0.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
171	0.5	187	8.3	203	2.1
172	0.5	188	1.4	204	2.6
173	2.4	189	1.2	205	13.1
174	2.4	190	0.2	206	2.1
175	1.2	191	0.2	207	0.5
176	0.7	192	0.5	218	0.2
177	2.9	193	0.2	219	0.2
179	0.7	200	0.2	220	2.1
181	0.2	201	0.2	221	0.7
186	0.2	202	9.3		

M(220) = 2.1

Base peak: at m/e 43

Molecular formula: $C_{15}H_{23}OH$

Double bond equivalents: four

Metastable ions:

Diffuse peaks	Possible break-down reactions
71.5	$121^+ \longrightarrow 93^+ + 28$
37.1	$121^+ \longrightarrow 67^+ + 54$
69.5	$119^+ \longrightarrow 91^+ + 28$
46.5	$91^+ \longrightarrow 65^+ + 26$
25.1	$129^+ \longrightarrow 57^+ + 72$
58.4	$107^+ \longrightarrow 79^+ + 28$
59.5	$105^+ \longrightarrow 79^+ + 26$
89.0	$93^+ \longrightarrow 91^+ + 2$
66.4	$93^+ \longrightarrow 79^+ + 14$
39.1	$83^+ \longrightarrow 57^+ + 26$
24.4	$69^+ \longrightarrow 41^+ + 28$

Ultraviolet data for a mixture of Fraction 30 and 31:

20.4 mg/100 ml

Shoulder absorbance started from λ 360 to 220 mμ.

Infrared data for a mixture of Fraction 30 and 31:

3450 (s, broad), 3050 (w), 2940 (s, broad), 2860 (s), 1730 (m), 1670 (w), 1635 (w), 1455 (sh), 1445 (m), 1380 (m), 1365 (m), 1275 (w), 1235 (w), 1180 (w), 1135 (m), 1120 (sh), 1080 (w), 1000 (m), 975 (w), 895 (m), 865 (m), 765 (sh), 755 (m) cm^{-1}.

Discussion and conclusions

The highest mass ion in the spectrum (m/e 220) may give rise to the following fragment ions:

m/e 220 ⟶ m/e 205 −15 (CH_3·)

⟶ m/e 203 −17 (OH·)

⟶ m/e 202 −18 (H_2O)

⟶ m/e 177 −43 (C_3H_7·)

The m/e 220-H_2O ion may give rise to the following fragment ions:

m/e 202 ⟶ m/e 187 −15 (CH_3·)

⟶ m/e 159 −43 (C_3H_7·)

The small peak at m/e 204 which can not be derived from m/e 220 suggested the presence of higher component than 220. The small peak at m/e 61 suggested acetate ester.

The infrared spectrum confirmed the presence of hydroxyl group by the broad absorption band at 3450 cm^{-1}. The small absorption bands at 1730 and 1635 cm^{-1} may be attributed to an acetate ester.

The elemental composition $C_{15}H_{24}O$ for m/e 220 could be

deduced by tracing the homologous series of ions.

The general appearance of the mass spectrum and the metastable ions of this fraction were closely related to those of the sesquiterpene hydrocarbon in Fraction 21. The mass spectra of Fractions 21 and 30 were very similar below m/e 119. The intense peaks higher than m/e 119 were two mass units lower, in the mass spectrum of 30, than the corresponding ones in the mass spectrum of 21, i.e. 131-133, 145-147, 159-161, 187-189 and 202-204.

In conclusion, the major component of Fraction 30 is probably a sesquiterpene alcohol of the cadinane type.

Bicyclic sesquiterpene alcohols of the formula $C_{15}H_{24}O$ are of common occurrence in ethereal oils (243).

Fractions 22, 24, 25, 26 and 27

The general appearance of the mass spectra of these fractions was similar but not identical.

At the low mass end the usual clusters of peaks 14 mass units apart indicated the presence of alkyl hydrocarbon moiety. The most intense peaks were at m/e 43, 55, 69, 81, 93 and 109. The peak at m/e 43 was either the base peak or the second most intense peak. There were distinct peaks at m/e 143, and either 125, 127 or 126.

At the high mass end the peaks were very small and the number of carbons could not be deduced.

The presence of oxygen was indicated in all components of these fractions.

The mass spectrum of Fraction 22 showed a 2 mass-unit shift in the intense peaks at m/e 69, 81 and 109, from the spectra of the other fractions. The peaks at m/e 27 and 99 were also more intense in the spectrum of this fraction than the corresponding peaks in Fractions 24, 25, 26 and 27.

Fraction 22

Results

Gas-liquid chromatography: this fraction had a large symmetric peak, a retention time of 22.8 mins., and represented 3.16% of the essential oil. The peak was augmented by geraniol. The collected colorless viscid liquid had a faint odor.

Mass spectral data: one scan was run on MS12 and one on MS9.

Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	1.1	31	3.3	47	0.4
13	0.7	37	3.0	48	0.7
14	3.3	38	5.2	49	4.1
15	8.9	39	42.6	50	17.4
16	0.4	40	8.1	51	28.5
19	0.4	41	50.0	52	8.9
26	13.3	42	15.2	53	21.1
27	100.0	43	83.3	54	10.0
28	30.0	44	3.7	55	76.3
29	27.0	45	3.0	56	24.8
30	0.7	46	0.4	57	12.6

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
58	3.0	84	4.1	111	47.8
59	1.1	85	4.8	112	3.7
60	0.7	86	0.9	113	1.5
61	2.6	87	0.6	114	0.7
62	5.2	88	0.4	115	0.7
63	7.8	89	2.2	116	0.7
64	1.9	90	2.6	117	1.1
65	10.4	91	5.6	118	0.4
66	4.4	92	1.5	119	2.6
67	60.7	93	7.8	120	0.7
68	9.6	94	1.5	121	0.7
69	13.3	95	4.1	122	0.4
70	5.6	96	1.5	123	0.7
71	28.9	97	3.3	124	0.4
72	2.2	98	11.5	125	1.9
73	1.9	99	16.3	126	9.6
74	2.2	100	1.5	127	3.0
75	2.2	101	1.1	128	1.1
76	0.4	103	0.7	129	0.7
77	13.7	104	0.4	130	0.4
78	3.0	105	2.6	131	1.5
79	54.8	106	0.7	132	0.4
80	7.0	107	10.0	133	1.5
81	14.8	108	4.8	134	0.7
82	7.4	109	3.7	135	1.5
83	17.4	110	2.2	136	0.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
137	1.5	159	0.4	179	0.4
139	0.4	160	0.4	180	0.4
140	0.4	161	0.4	181	0.4
141	1.1	162	0.4	183	0.2
142	0.4	163	0.7	184	0.4
143	0.7	165	0.7	185	0.4
144	0.4	166	0.6	187	0.4
145	2.6	167	0.4	189	0.4
146	0.6	168	0.6	191	0.4
147	0.7	169	0.4	193	0.4
148	0.4	170	0.2	194	0.4
151	0.4	171	0.4	196	0.4
152	0.4	173	0.6	201	0.2
154	0.4	174	0.2	202	0.6
155	0.4	175	0.4	203	0.4
156	0.4	176	0.4	204	0.6
157	0.4	177	0.6	205	0.7
158	0.4	178	0.2	206	0.4
				207	0.4

Base peak: at m/e 27

Metastable ions:

Diffuse peaks	Possible break-down reactions
25.1	$67^+ \longrightarrow 41^+ + 26$
97.6	$126^+ \longrightarrow 111^+ + 15$

Data from MS9:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	44	2.9	72	3.3
13	0.3	45	1.4	73	0.8
14	2.0	49	4.8	74	2.4
15	11.0	50	15.2	75	1.0
16	0.8	51	23.8	76	0.5
17	5.7	52	7.1	77	10.0
18	23.3	53	14.3	78	1.9
19	0.4	54	9.3	79	54.8
20	0.2	55	83.3	80	3.3
26	12.4	56	38.0	81	9.0
27	71.4	57	3.8	82	5.2
28	52.4	58	3.1	83	31.0
29	23.8	59	0.9	84	4.8
30	0.6	60	0.8	85	2.4
31	2.4	61	3.3	86	0.8
32	8.6	62	4.8	87	0.7
33	0.3	63	6.2	88	0.3
34	0.7	64	1.1	89	2.9
37	3.3	65	1.0	90	3.3
38	4.3	66	3.3	91	3.8
39	42.9	67	66.7	92	0.8
40	6.7	68	9.0	93	3.8
41	40.5	69	4.8	94	1.4
42	9.0	70	5.0	95	1.9
43	100.0	71	50.0	96	1.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
97	3.3	123	0.8	149	0.4
98	23.8	124	0.7	150	0.6
99	31.0	125	1.3	151	0.4
100	2.1	126	31.0	152	0.3
101	0.7	127	3.1	153	0.7
102	0.3	128	1.1	154	0.2
103	0.5	129	0.7	155	0.3
104	0.2	130	0.3	157	0.4
105	1.7	131	1.0	158	0.4
106	0.7	132	0.4	159	5.2
107	11.4	133	0.7	160	1.0
108	4.5	134	0.3	161	0.3
109	2.1	135	0.6	162	0.1
110	2.4	136	0.3	163	0.6
111	97.6	137	0.6	164	0.2
112	6.7	138	0.3	165	0.8
113	1.4	139	0.3	166	0.2
114	0.4	140	0.3	167	0.3
115	0.6	141	0.4	168	0.5
116	0.3	142	0.4	169	0.3
117	0.8	143	0.7	171	0.1
118	0.4	144	0.6	173	0.4
119	0.8	145	1.3	174	0.2
120	0.3	146	0.2	175	0.2
121	0.8	147	0.4	176	0.2
122	0.4	148	0.3	177	0.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
178	0.3	195	0.1	216	0.1
179	0.5	196	0.1	217	0.1
180	0.1	198	0.1	218	0.2
181	0.1	199	0.1	219	0.1
182	0.1	200	0.2	220	0.4
183	0.2	201	0.2	221	0.5
184	0.2	202	0.7	222	0.2
185	0.2	203	0.3	223	0.1
187	0.3	204	0.2	232	0.1
188	0.2	205	0.5	233	0.1
189	0.2	206	0.1	234	0.1
190	0.1	207	0.1	235	0.1
191	0.1	208	0.1	236	1.1
192	0.1	209	0.1	237	0.2
193	0.8	210	0.1	238	0.1

An accurate mass determination of the molecular ion (m/e 236) was made:

measured 236.1774; calculated for $C_{15}H_{24}O_2$: 236.1776.

M(236) = 1.1

Base peak: at m/e 43

Molecular formula: $C_{15}H_{24}O_2$

Double bond equivalents: four

Ultraviolet data: 11 mg/100 ml

λ_{max}^{EtOH}	E_{max}
227	360
285	5

Infrared data:

3445 (m, broad), 3250 (m), 3080 (w), 2970 (sh), 2930 (s), 2860 (sh), 1775 (s, broad), 1680 (m), 1640 (w), 1455 (m), 1410 (m), 1375 (m, sharp), 1285 (w), 1260 (w), 1225 (m), 1195 (m), 1130 (s), 1080 (w), 1030 (w), 985 (w), 955 (w), 925 (m), 790 (w) cm^{-1}.

An infrared spectrum of an authentic sample of geraniol, $C_{10}H_{18}O$, was recorded:

3320 (s, broad), 2960 (s), 2910 (s), 2860 (sh), 1665 (m), 1440 (s), 1375 (s), 1230 (w), 1090 (m), 990 (s, broad), 815 (w), 730 (w) cm^{-1}.

Discussion and conclusions

The retention data originally suggested geraniol. This was ruled out on comparison of the infrared spectrum of this fraction with that of an authentic sample of geraniol. The mass spectra comparison also ruled out geraniol (225).

The high-resolution mass spectrometry established the formula $C_{15}H_{24}O_2$ for m/e 236 which is probably the molecular ion of the major component of this fraction. The small peaks at m/e 221 and 218 may be derived from it by the loss of a methyl radical and a water molecule. The small peaks at m/e 222 and 220, which can not be derived from m/e 236, may be minor components of Fraction 22.

Both spectra obtained from MS12 and MS9 were very similar. Differences between the two spectra may be summarized.

The mass spectrum recorded on MS12 was lacking the

molecular ion (m/e 236) and the distinct peak at m/e 159. The base peak was at m/e 27, the second most intense peak at m/e 43, and the m/e 111 peak was the sixth most intense.

The base peak in the mass spectrum recorded on MS9 was at m/e 43, while the m/e 111 and 27 peaks were the second and fourth most intense. The base peak was shown by the high-resolution to be approximately equally split indicating that both C_3H_7+ and CH_3CO+ were equally abundant.

The base peak (m/e 27), which corresponds to C_2H_3+, was suggested to be the result of cleavages such as (244): CH_2=CH$\}$R, CH_2=CH$\}$CO-R, or CH_2=CH$\}$COOR. It indicates compounds containing alkenyl groups (245). The C=C stretching at 1640 cm^{-1}, and the two bands at 985 and 925 cm^{-1}, and the C-H stretching at 3080 cm^{-1}, in the infrared spectrum, confirmed the presence of vinylic group.

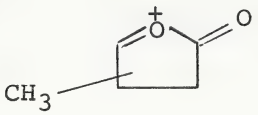
The infrared spectrum and the intensity of the molecular ion excluded aromaticity.

The strong broad absorption band at 1775 cm^{-1} in the infrared spectrum suggested either cyclobutanone or γ-lactone. The strong C-O stretching absorption at 1130 cm^{-1} confirmed γ-lactone.

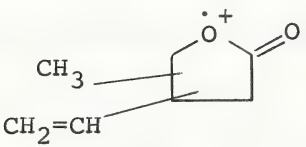
The intense peak at m/e 99 in the mass spectra suggested δ-lactone (246,247). The mass spectra of γ-lactones show intense peak at m/e 85.

As both the infrared and the mass spectra suggested lactone and the difference was 14 mass units in the mass spectra of γ- and δ-lactones, it is probable that the

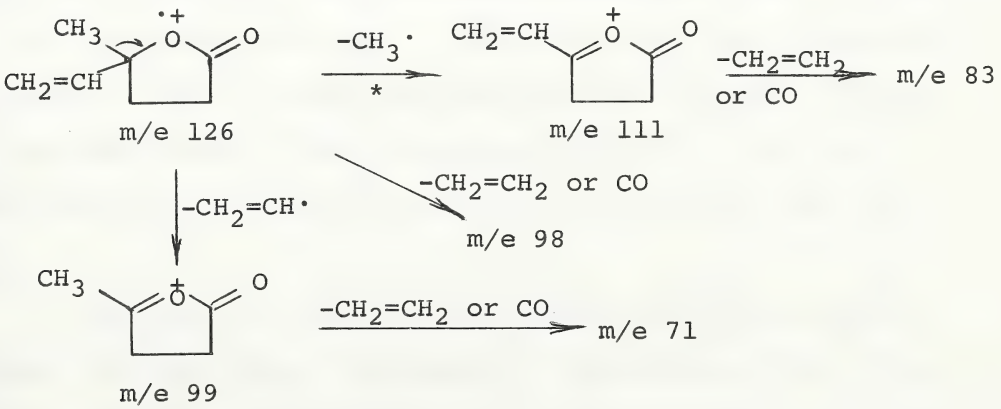
γ-lactone in this fraction bears a methyl substituent on the lactone ring. The following structure may be proposed for m/e 99:



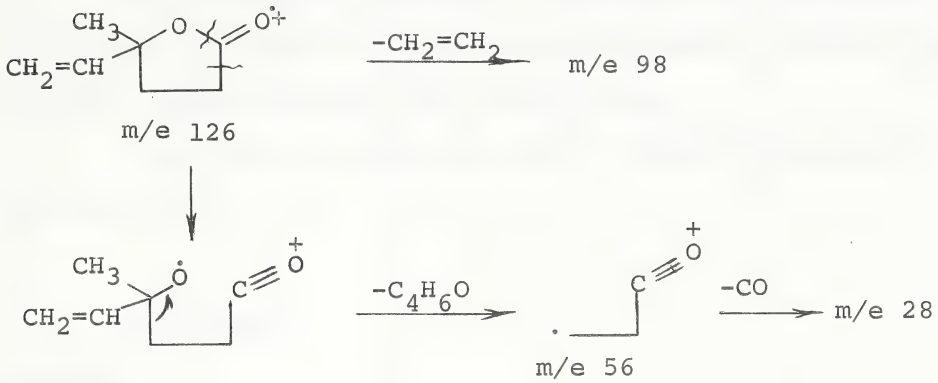
The m/e 111 and 99 fragments seem to be derived from the odd-electron ion m/e 126 by the loss of a methyl and a vinyl radical. There is a suitable metastable indicating the transition: $126^{+} \longrightarrow 111^{+} + 15$. Tentatively the following structure may be assigned to m/e 126:



The position of the methyl and vinyl groups may be assigned adjacent to the oxygen which facilitates their expulsion (247). The following structure is used tentatively:



The charge may also be localized on the carbonyl group as follows (248):



The m/e 126 fragment is derived probably from the molecular ion by the loss of 110 mass units:

$$[C_{15}H_{24}O_2]^{+\cdot} \xrightarrow{-C_8H_{14}} [C_7H_{10}O_2]^{+\cdot}$$

m/e 236 m/e 126

There was a small peak in the mass spectra at m/e 110 which seemed to lose successive hydrogen atoms to yield a distinct peak at m/e 107. However m/e 107 may be explained also as being derived from m/e 111 by successive loss of 4 hydrogen atoms. These successive loss of 4 mass units was obvious in the peaks at m/e 111, 107, 83, 79, and 71, 67.

The rest of the molecule, beside the methyl substituted γ-lactone ring, may contain a cyclohexane ring. This was suggested by the presence of an intense peak at m/e 55

with no complimentary intense M-55 peak (236). The intense peak at m/e 79 also suggested polycycloalkane (249).

In conclusion, the major component of Fraction 22 ($C_{15}H_{24}O_2$) is believed to be a sesquiterpene γ-lactone with a methyl substituent on the lactone ring.

Sesquiterpene lactones are of common occurrence in volatile oils (111).

Fraction 24

Results

Gas-liquid chromatography: this fraction had a small peak, a retention time of 25.5 mins., and represented 1.95% of the essential oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	31	3.3	48	0.3
13	0.2	37	1.0	49	0.9
14	1.3	38	0.7	50	2.8
15	3.3	39	23.3	51	4.8
19	0.5	40	5.5	52	2.8
20	0.1	41	74.2	53	17.8
24	0.2	42	9.0	54	4.5
25	0.7	43	100.0	55	44.8
26	3.0	44	4.0	56	7.8
27	23.0	45	4.2	57	18.0
29	25.2	46	0.2	58	8.8
30	0.7	47	0.2	59	4.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
60	0.7	86	1.5	112	2.3
61	0.2	87	1.2	113	2.1
62	0.7	88	1.2	115	1.3
63	2.3	89	0.9	116	0.7
64	0.6	90	0.3	117	1.5
65	4.7	91	9.7	118	0.7
66	3.3	92	2.5	119	4.5
67	22.5	93	22.8	120	2.7
68	14.5	94	6.5	121	3.3
69	56.0	95	11.0	122	1.3
70	7.1	96	7.1	123	5.1
71	24.2	97	6.0	124	1.7
72	4.7	98	2.5	125	8.0
73	2.0	99	5.3	126	8.8
74	1.7	100	1.8	127	10.0
75	0.8	101	1.5	128	2.3
76	0.2	102	0.5	129	1.2
77	10.3	103	1.3	130	0.5
78	3.5	104	1.7	131	1.8
79	14.2	105	6.2	132	0.9
80	4.2	106	1.8	133	2.8
81	26.0	107	8.7	135	3.3
82	8.3	108	13.2	136	1.2
83	16.3	109	33.0	137	2.1
84	5.2	110	2.1	138	9.5
85	12.8	111	19.5	139	1.5

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
140	0.9	167	0.5	194	0.7
141	1.7	168	0.5	195	1.7
142	1.4	169	0.5	196	0.3
143	6.7	170	0.5	197	0.2
144	0.7	171	0.5	198	0.2
145	2.1	172	0.2	199	0.2
146	0.7	173	0.7	200	0.5
147	1.8	174	0.2	201	0.2
148	1.0	175	1.0	202	0.7
150	0.9	176	0.5	203	0.5
151	4.0	177	0.7	204	1.3
152	1.5	178	0.3	205	1.0
153	1.2	179	0.7	206	0.3
154	0.7	180	0.3	207	0.5
155	2.2	181	0.7	208	0.5
156	0.7	182	0.3	209	0.3
157	2.0	183	0.3	210	0.2
158	0.5	184	0.4	217	0.2
159	1.8	185	0.3	218	0.3
160	0.5	187	0.3	219	0.5
161	2.0	188	0.2	220	0.7
162	1.7	189	0.3	221	0.7
163	0.9	190	0.3	222	0.2
164	0.5	191	0.3	223	0.2
165	0.9	192	0.2	224	0.2
166	0.5	193	0.7	225	0.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
236	0.3	238	0.3	240	0.2
237	0.5	239	0.3		

M(238) = 0.3

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
41.2	$238^+ \longrightarrow 99^+ + 139$
63.7	$136^+ \longrightarrow 93^+ + 43$
37.1	$121^+ \longrightarrow 67^+ + 54$
89.0	$93^+ \longrightarrow 91^+ + 2$
66.4	$93^+ \longrightarrow 79^+ + 14$
24.3	$69^+ \longrightarrow 41^+ + 28$

Discussion and conclusions

The mass spectrum of this fraction was closely related to the mass spectra of Fractions 25 and 26 which were recorded on MS12.

The highest even-mass ion (m/e 238) may be the molecular ion. The molecular formula could not be deduced.

The only conclusion which could be derived from the general appearance of the mass spectrum is that Fraction 24 may contain an oxygenated sesquiterpene.

Fraction 25

Results

Gas-liquid chromatography: this fraction had a sharp symmetric peak, a retention time of 26.6 mins., and represented 4.67% of the essential oil. The peak was augmented by safrole. The collected viscid liquid when tested on Se-30 column gave a single sharp peak indicating its purity.

Mass spectral data: three different scans were run on MS12 and one on MS9.

Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.1	31	4.0	52	2.8
13	0.2	37	0.6	53	23.7
14	0.7	38	1.2	54	3.6
15	2.9	39	26.2	55	45.8
16	0.2	40	6.8	56	4.8
17	0.2	41	100.0	57	9.2
18	0.3	42	8.2	58	7.2
19	0.3	43	91.5	59	9.2
20	0.1	44	2.6	60	0.4
24	0.2	45	4.9	62	0.4
25	0.5	46	0.1	63	1.3
26	1.9	47	0.1	64	0.4
27	22.7	48	0.1	65	6.8
28	2.2	49	0.3	66	3.4
29	20.2	50	1.4	67	26.8
30	0.5	51	4.5	68	12.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
69	72.9	95	9.2	123	3.2
70	54.5	96	4.5	124	1.0
71	22.2	97	4.9	125	9.2
72	3.4	100	0.4	126	2.8
73	2.3	101	0.6	127	6.2
74	1.3	102	0.2	128	1.0
75	0.6	103	0.8	133	3.5
76	0.1	104	0.4	135	3.5
77	11.3	105	6.0	137	1.4
78	2.5	106	1.3	138	4.0
79	16.0	107	11.5	139	0.9
80	3.8	108	4.9	140	0.4
81	24.5	109	30.7	141	0.4
82	6.8	110	4.9	142	0.5
83	11.4	111	10.4	143	13.8
84	3.8	112	1.4	144	1.4
85	11.5	113	1.4	145	1.0
86	0.8	114	0.4	146	0.7
87	0.6	115	1.1	147	1.4
88	1.2	116	0.3	148	1.1
89	0.6	117	1.1	149	0.9
90	0.3	118	0.4	150	0.6
91	11.2	119	4.8	151	2.4
92	3.8	120	3.8	152	1.4
93	34.5	121	3.4	153	0.7
94	7.5	122	0.6	154	1.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
155	1.8	176	0.1	205	0.6
156	0.4	177	0.3	206	0.1
157	0.2	178	0.1	207	0.1
158	0.1	179	0.1	208	0.1
159	0.6	180	0.1	209	0.1
160	0.2	181	0.2	211	0.1
161	1.8	185	0.1	218	0.1
162	0.5	187	0.2	220	0.1
163	0.3	188	0.1	221	0.3
164	0.1	189	0.4	222	0.3
165	0.3	190	0.1	223	0.1
166	0.3	191	0.1	224	0.1
167	0.1	193	0.1	235	0.2
169	0.1	195	1.0	236	0.3
170	0.3	196	0.4	237	0.2
171	0.1	197	0.1	238	0.4
173	0.1	202	0.1	239	0.3
174	0.1	203	0.1	240	0.1
175	0.4	204	1.3		

M(238) = 0.4

Base peak: at m/e 41

Metastable ions:

Diffuse peaks	Possible break-down reactions
36.5	$138^+ \longrightarrow 71^+ + 67$
14.8	$125^+ \longrightarrow 43^+ + 82$
66.5	$93^+ \longrightarrow 79^+ + 14$
24.5	$69^+ \longrightarrow 41^+ + 28$
25.1	$67^+ \longrightarrow 41^+ + 26$

Data from MS9:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.1	43	96.0	68	13.0
13	0.1	44	3.0	69	96.0
14	0.5	45	3.6	70	10.0
15	1.4	46	0.1	71	31.0
16	0.3	47	0.2	72	2.5
17	3.3	48	0.1	73	0.7
18	16.0	49	0.1	74	3.1
19	0.3	50	0.5	75	0.3
20	0.4	51	1.4	76	0.1
25	0.1	52	0.9	77	4.5
26	0.7	53	12.0	78	0.9
27	12.0	54	2.2	79	9.0
28	22.0	55	28.0	80	2.2
29	11.0	56	3.0	81	22.0
30	0.3	57	2.6	82	9.6
31	2.0	58	8.0	83	16.0
32	3.9	59	3.9	84	3.5
33	0.2	60	0.2	85	5.8
34	0.5	61	0.2	86	1.6
37	0.3	62	0.1	87	1.6
38	0.3	63	0.3	88	4.2
39	12.0	64	0.1	89	0.3
40	3.0	65	2.8	91	4.2
41	63.0	66	1.4	92	1.1
42	4.0	67	22.0	93	33.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
94	13.0	120	1.6	147	0.6
95	10.0	121	1.9	148	0.2
96	8.0	122	0.6	149	0.8
97	7.0	123	3.8	150	0.2
98	1.4	124	0.7	151	5.6
99	1.1	125	3.8	152	2.8
100	0.4	126	5.8	153	1.0
101	0.4	127	17.0	154	2.1
102	0.1	128	2.0	155	6.0
103	0.3	129	0.5	156	1.3
104	0.3	131	0.6	157	0.3
105	2.3	132	0.2	158	0.1
106	0.5	133	1.5	159	0.5
107	4.4	134	0.5	160	0.1
108	14.0	135	2.8	161	0.4
109	100.0	136	0.7	162	0.2
110	12.0	137	0.3	163	0.3
111	27.0	138	17.0	164	0.3
112	3.3	139	2.8	165	0.3
113	1.3	140	0.4	166	0.2
114	0.2	141	0.4	167	0.2
115	0.3	142	0.3	168	0.3
116	0.1	143	4.2	169	0.1
117	0.5	144	0.5	170	1.1
118	0.1	145	0.6	171	0.2
119	1.8	146	0.2	173	0.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
175	0.3	193	0.2	218	0.1
176	0.1	194	0.1	220	0.8
177	1.2	195	0.7	221	0.3
178	0.2	196	0.2	222	0.1
179	0.1	202	0.1	223	1.0
180	0.2	203	0.1	224	0.2
181	0.1	204	0.1	236	0.1
187	0.7	205	0.8	237	0.1
188	0.1	206	0.1	238	0.6
189	0.1	209	0.1	239	0.2
191	0.1	211	0.2		

An accurate mass measurement of the molecular ion (m/e 238) was made:

measured 238.1933; calculated for $C_{15}H_{26}O_2$: 238.1933.

M(238) = 0.6

Base peak: at m/e 109

Molecular formula: $C_{15}H_{26}O_2$

Double bond equivalents: three

Ultraviolet data: 10 mg/100 ml

λ_{max}^{EtOH}	E_{max}
230	238
280	24

Infrared data (a spacer was used):

3460 (m, broad), 3080 (w), 2960 (s), 2920 (s), 2860 (sh), 1725 (w), 1640 (w), 1445 (m), 1365 (m), 1280 (w), 1170 (sh), 1115 (m), 1090 (sh), 1045 (w), 1015 (w), 980 (sh), 905 (m) cm^{-1}.

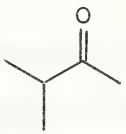
An infrared spectrum of an authentic sample of safrole, $C_{10}H_{10}O_2$, was recorded:
3070 (m, sharp), 3000 (sh), 2970 (m, sharp), 2880 (s), 2760 (m, sharp), 1840 (m), 1730 (m), 1635 (s), 1605 (m), 1490 (s), 1435 (s), 1405 (sh), 1350 (s), 1275 (m), 1235 (s), 1195 (sh), 1175 (s), 1115 (m), 1085 (m), 1030 (s), 985 (m), 910 (s, broad), 845 (m), 795 (s), 765 (s), 710 (sh), 705 (w), 650 (m) cm^{-1}.

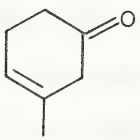
Discussion and conclusions

The retention data suggested safrole, but on comparison of the infrared spectra this was ruled out.

The precise mass measurement made with MS9 indicated the formula $C_{15}H_{26}O_2$ for the molecular ion (m/e 238).

The ultraviolet data suggested monounsaturated chromophore (237). It also suggested chromophores such as (250, 251):





The second most intense peak (m/e 43) in the mass spectra was shown by the high-resolution to be split. The larger split was at lower mass indicating CH_3CO^+. This, together with the rearrangement peak at m/e 58, suggested a ketone.

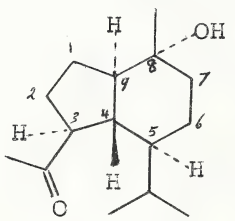
The infrared showed a broad hydroxyl absorption band in the region 3300-3600 cm^{-1}, and a broad band at 1115 cm^{-1} which may be due to C-O stretching. The small m/e 220 peak

in the mass spectrum (MS9) may be due to M-H_2O ion.

These data suggested that $C_{15}H_{26}O_2$ may be a sesquiterpene ketone and secondary alcohol. There was no strong carbonyl absorption in the region 1696-1750 cm^{-1} of the infrared spectrum. However, the infrared spectrum was poorly resolved because of the small amount of the sample.

A sesquiterpene alcohol of the same molecular formula, oplopanone, has been isolated by Takeda, Minato and Ishikawa (73). The structure and absolute configuration of oplopanone have been reported by them (73):

Oplopanone



Oplopanone is believed to belong to a modified cadinane type sesquiterpene (252). The precurser of oplopanone in the plant body is believed to be α-cadinol (73).

For comparison, an authentic sample of oplopanone has been kindly sent by Dr. Ken'ichi Takeda, Shionogi Research Laboratory, Japan.

The mass spectrum of oplopanone was recorded on A.E.I. MS9. The result is shown in figure 2. The mass spectrum of oplopanone was not similar to that of Fraction 25. This dissimilarity was thought to be due to the difference in the methods of isolation. Separation by gas-liquid chromato-

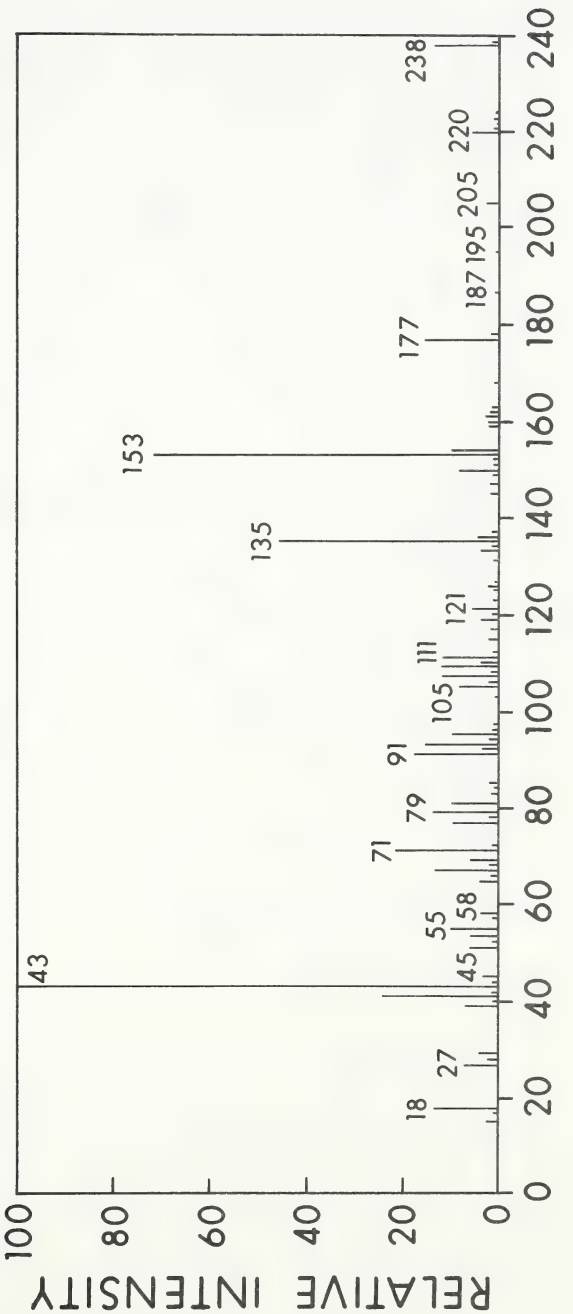


Figure 2. Mass Spectrum of Oplopanone (MS9)

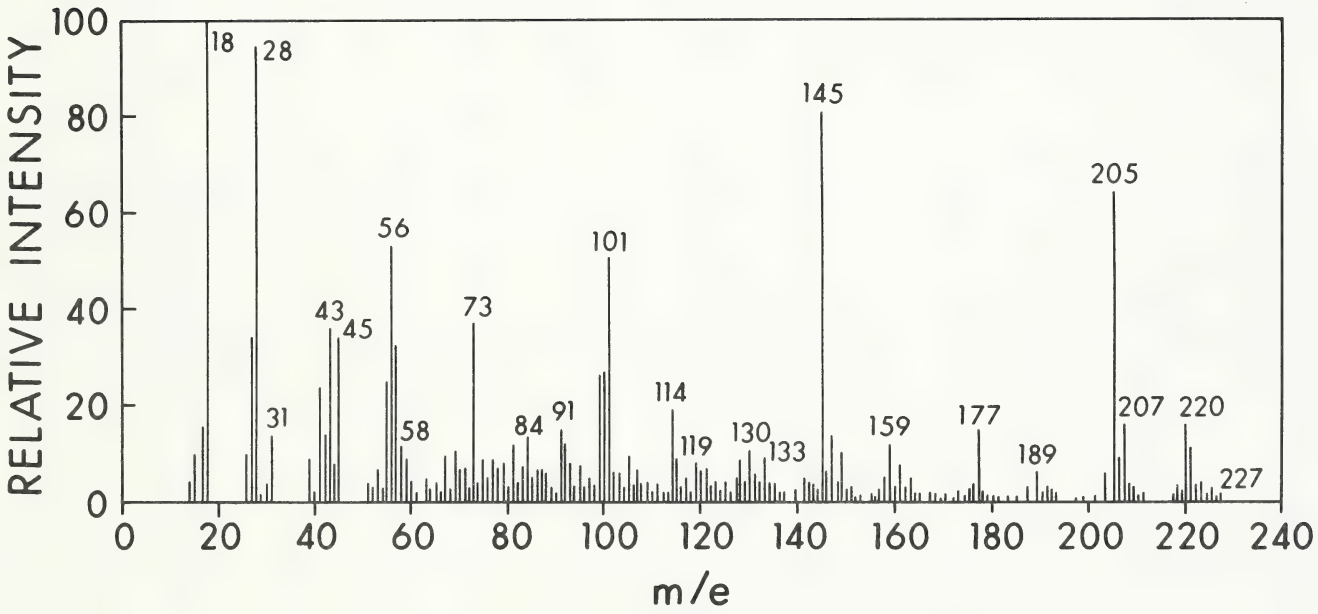


Figure 3. Mass Spectrum of Oplopanone After Passing Through Lac-728 Column (MS12)

graphy might have caused changes in structure. It was decided to pass a sample of oplopanone through the Lac-728 column and record the mass spectrum of the effluent on A.E.I. MS12. The result is shown in figure 3.

The five scans of oplopanone run on MS12 were similar to each other, but different from the two scans run on MS9, indicating change in structure.

Comparison of the mass spectra of Fraction 25 with those of oplopanone indicated that this fraction does not contain oplopanone.

In summary, Fraction 25 contains an oxygenated sesquiterpene of the formula $C_{15}H_{26}O_2$. No definite conclusions could be derived about its structure. Change in structure during separation by gas-liquid chromatography is probable.

Fraction 26

Results

Gas-liquid chromatography: this fraction had a very large symmetric peak, a retention time of 27.6 mins., and represented 11.84% of the essential oil. The collected viscid liquid when tested for purity on Se-30 gave a broad peak which was split at the top.

Mass spectral data: three different scans were run on MS12 and another three on MS9.

Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	47	0.1	72	5.2
13	0.2	48	0.1	73	5.6
14	0.8	49	0.3	74	0.8
15	4.1	50	1.3	75	0.6
16	0.3	51	3.3	76	0.2
17	0.4	52	2.1	77	8.1
18	1.6	53	19.0	78	2.4
19	0.3	54	3.8	79	12.5
20	0.1	55	47.7	80	4.7
24	0.2	56	4.6	81	26.0
25	0.4	57	12.8	82	7.3
27	20.2	58	4.7	83	11.2
28	5.3	59	13.4	84	5.2
29	19.3	60	0.4	85	19.5
30	0.5	61	0.5	86	1.3
31	6.7	62	0.3	87	0.5
37	0.7	63	1.0	88	0.4
39	20.7	64	0.5	89	0.5
40	5.6	65	4.7	90	0.2
41	78.0	66	3.0	91	8.1
42	7.6	67	26.0	92	2.1
43	100.0	68	27.8	93	26.0
44	4.2	69	52.1	94	4.7
45	4.7	70	6.5	95	8.0
46	0.1	71	36.9	96	3.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
97	6.0	130	0.2	156	0.4
98	1.7	131	0.9	157	0.4
100	0.4	132	0.4	158	0.1
101	1.1	133	2.5	159	0.4
102	0.4	134	2.1	160	0.4
103	0.7	135	3.9	161	3.9
104	0.4	136	3.5	162	0.8
105	6.0	137	2.1	163	0.4
106	1.7	138	2.0	164	0.3
107	13.9	139	0.8	165	0.1
108	3.9	140	0.5	166	0.2
109	16.5	141	0.5	167	0.1
110	3.0	142	1.0	168	0.1
111	7.3	143	32.6	169	0.1
113	2.1	144	2.4	170	0.2
119	5.6	145	0.8	171	0.1
120	2.1	146	0.2	173	0.2
121	4.7	147	0.8	175	0.3
122	4.3	148	0.8	176	0.2
123	12.4	149	0.9	177	0.5
124	2.1	150	0.3	178	0.2
125	3.9	151	2.6	179	0.4
126	2.1	152	0.4	180	0.1
127	3.9	153	0.4	181	0.2
128	0.9	154	0.4	185	0.2
129	0.6	155	0.8	186	0.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
187	0.2	203	0.2	221	0.4
189	0.7	204	1.3	222	0.2
190	0.1	205	0.4	223	0.2
192	0.1	206	0.1	236	0.2
193	0.1	207	0.1	237	0.1
195	2.0	209	0.1	238	0.1
196	0.5	218	0.1	239	0.9
197	0.1	220	0.2	240	0.1

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
28.6	$254^+ \longrightarrow 85^+ + 169$
24.5	$254^+ \longrightarrow 79^+ + 175$
63.5	$254^+ \longrightarrow 127^+ + 127$
75.5	$79^+ \longrightarrow 77^+ + 2$
84.3	$136^+ \longrightarrow 107^+ + 29$
63.5	$136^+ \longrightarrow 93^+ + 43$
37.1	$121^+ \longrightarrow 67^+ + 54$
58.3	$107^+ \longrightarrow 79^+ + 28$
13.7	$53^+ \longrightarrow 27^+ + 26$

Data from MS9:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.1	16	0.3	20	0.1
13	0.1	17	5.3	27	6.9
14	0.3	18	50.0	28	9.7
15	1.2	19	0.2	29	6.9

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
30	0.2	65	1.9	91	3.8
31	2.5	66	0.8	92	1.5
32	2.2	67	11.3	93	50.0
39	6.3	68	6.3	94	5.0
40	1.9	69	100.0	95	5.6
41	75.0	70	4.4	96	1.6
42	2.5	71	56.2	97	2.8
43	96.9	72	3.1	98	0.9
44	1.3	73	3.4	99	0.7
45	1.9	74	0.6	100	0.2
46	0.1	75	0.2	101	0.8
50	0.3	76	0.1	102	0.2
51	0.9	77	3.4	103	0.2
52	0.5	78	0.6	104	0.1
53	6.9	79	7.5	105	2.5
54	1.1	80	4.1	106	0.7
55	16.3	81	15.3	107	10.3
56	1.9	82	5.6	108	4.1
57	2.8	83	5.9	109	46.9
58	2.5	84	3.4	110	2.8
59	5.6	85	11.3	111	7.2
60	0.2	86	0.8	112	1.0
61	0.2	87	0.3	113	1.9
62	0.1	88	0.5	114	0.4
63	0.2	89	0.2	115	0.2
64	0.1	90	0.1	116	0.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
117	0.3	143	56.3	170	0.2
118	0.2	144	2.8	171	0.1
119	3.8	145	0.6	173	0.1
120	1.4	146	0.1	175	0.2
121	3.8	147	0.9	176	0.2
122	1.3	148	0.7	177	0.4
123	3.8	149	0.7	178	0.2
124	0.7	150	0.3	179	0.2
125	9.4	151	1.8	180	0.1
126	2.5	152	0.7	181	0.1
127	4.4	153	0.5	185	0.1
128	0.6	154	0.4	187	0.1
129	0.2	155	1.1	189	0.8
130	0.1	156	0.2	190	0.1
131	0.2	157	0.1	191	0.1
132	0.2	159	0.5	193	0.1
133	2.5	160	0.1	195	1.5
134	1.5	161	3.4	196	0.5
135	3.8	162	0.8	197	0.1
136	4.7	163	0.2	202	0.1
137	1.6	164	0.1	203	0.1
138	3.4	165	0.1	204	1.1
139	0.7	166	0.2	205	0.3
140	0.3	167	0.1	207	0.1
141	0.2	168	0.1	209	0.1
142	0.8	169	0.1	220	0.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
221	0.2	224	0.1	239	0.6
222	0.1	236	0.1	240	0.1
223	0.2	238	0.1		

An accurate mass determination of the following ions was made:

m/e 239: measured 239.1652; calculated for $C_{14}H_{23}O_3$: 239.1647.

m/e 204: measured 204.1874; calculated for $C_{15}H_{24}$: 204.1878.

m/e 161: measured 161.1332; calculated for $C_{12}H_{17}$: 161.1330.

Base peak: at m/e 69

m/e	Elemental composition
239	$C_{14}H_{23}O_3$
204	$C_{15}H_{24}$
161	$C_{12}H_{17}$

Ultraviolet data: 18 mg/100 ml

λ_{max}^{EtOH}	$E_{1\ cm}^{1\%}$
275	14
235(s)	24
225(s)	29

Infrared data:

3450 (m, broad), 3080 (w, sharp), 2970 (s), 2910 (s), 2860 (sh), 1730 (w), 1640 (w), 1445 (m), 1400 (w), 1365 (m), 1290 (w), 1170 (w), 1140 (sh), 1100 (m), 1050 (m), 1025 (sh), 985 (m), 940 (w), 910 (s), 825 (w) cm^{-1}.

Nuclear magnetic resonance data: figure 4, sweep width 500 cps, sweep offset 500 cps.

Discussion and conclusions

The high-resolution mass spectrometry indicated the formulas $C_{15}H_{24}$ and $C_{12}H_{17}$ for m/e 204 and 161.

The presence of oxygen was deduced from the peaks at m/e 31, 45, 59 and 73 in the mass spectra, and the broad hydroxyl absorption at 3450 cm^{-1} in the infrared spectrum.

It was obvious that m/e 204 was not the molecular ion of the major component of this fraction because of the lack of oxygen. The presence of peaks below m/e 204 which can not be derived from it pointed to the presence of higher component. To detect the molecular ion the sample was introduced by direct probe at 50°C to avoid decomposition in the inlet system.

The highest mass ion under these conditions was m/e 254 which could not be accurately measured. However the accurate mass determination of m/e 239 indicated the formula $C_{14}H_{23}O_3$.

Obviously m/e 204 can not be derived from m/e 239 indicating the presence of more than one component in Fraction 26. Assuming that m/e 239 was derived from m/e 254, by the loss of a methyl radical, the formula $C_{15}H_{26}O_3$ may be assigned to m/e 254. There were suitable metastables indicating the following transitions:

$$254^+ \longrightarrow 85^+ + 169$$

$$254^+ \longrightarrow 79^+ + 175$$

$$254^+ \longrightarrow 127^+ + 127$$

However there was no conclusive evidence that m/e 254 is

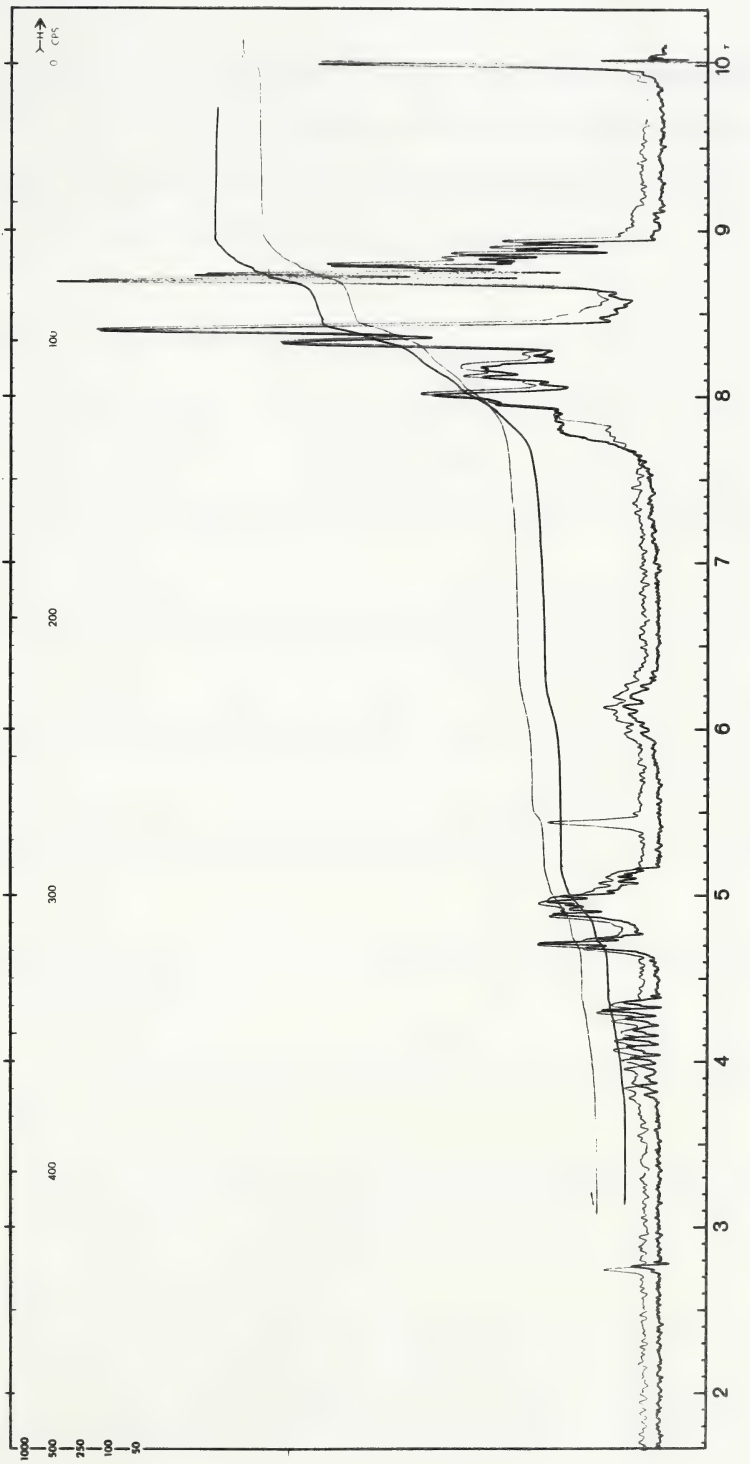


Figure 4. NMR Spectrum of Fraction 26

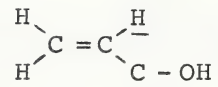
the molecular ion.

Although the molecular ion of the major component of this fraction was not definitely detected, some structural information could be deduced from the available data.

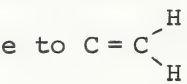
The NMR and infrared spectra showed lack of aromaticity.

The C-H and C=C stretching bands at 3080 and 1640 cm^{-1} indicated unsaturation. The two absorption bands at 910 and 985 cm^{-1} suggested out-of-plane bending due to terminal olefin.

The NMR spectrum (figure 4) confirmed the presence of vinylic protons by:

the broad multiplet at τ4-4.1 due to 

the triplet at τ4.7 due to

the complex signal at τ4.95 due to 

The intense peak at m/e 109 in the mass spectra also supported the diene (233).

The ultraviolet data suggested diunsaturated nonconjugated chromophore (237).

The hydroxyl protons could be detected in the NMR spectrum after shaking with deuterated water. The absorption at τ7.75 was removed to τ5.45.

Primary alcohol could be excluded by the absence of absorption at τ6.6, due to CH_2OH, in the NMR spectrum, and the absence of distinct M-18 and large m/e 31 peaks in the

mass spectra.

The m/e 59 peak being larger than the m/e 45 and 31 peaks suggested tertiary alcohol. The C-O band at 1100 cm^{-1} in the infrared spectrum may be attributed to cyclic tertiary alcohol. The complex absorption at τ8.7-8.8 in the NMR spectrum suggested cycloheptane.

The presence of a secondary alcohol was suggested by the NMR absorption at τ6.1-6.2. The C-O band at 1050 cm^{-1} in the infrared spectrum also suggested secondary α-unsaturated alcohol or secondary alicyclic five or six membered ring alcohol.

No definite conclusions could be derived from the complex signals in the NMR spectrum.

In the mass spectra the intense m/e 93 peak (C_7H_9+) is common in sesquiterpenes (233, 253). The split of m/e 43 peak, shown by high-resolution, indicated that CH_3CO+ was more abundant than C_3H_7+.

In conclusion, Fraction 26 contains more than one component. The molecular ion of the major component was not detected. The infrared, ultraviolet, NMR and mass spectra suggested oxygenated sesquiterpene, unsaturated, nonconjugated, probably having secondary and tertiary alcohol groups.

Fraction 27

Results

Gas-liquid chromatography: this fraction had a small peak which was not well resolved from 26, a retention time of 28.4 mins., and represented 4.28% of the essential oil.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.4	38	2.4	58	3.6
13	0.4	39	20.0	59	16.7
14	1.1	40	4.8	60	0.7
15	4.3	41	57.1	61	1.1
16	0.5	42	7.6	62	0.5
17	1.7	43	100.0	63	1.2
18	5.7	44	4.8	64	1.1
19	0.4	45	5.2	65	5.7
20	0.2	46	0.2	66	2.6
24	0.2	47	0.2	67	20.0
25	0.5	48	0.4	68	8.6
26	3.1	49	0.4	69	19.0
27	20.0	50	1.9	70	3.5
28	12.4	51	3.6	71	28.6
29	20.5	52	2.3	72	5.7
30	0.8	53	15.7	73	7.6
31	9.5	54	3.6	74	0.7
32	0.4	55	41.0	75	1.1
36	0.4	56	6.7	76	1.2
37	0.9	57	15.7	77	8.6

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
78	2.1	104	1.4	131	1.1
79	10.0	105	6.7	132	0.6
80	3.1	106	1.5	133	2.2
81	23.8	107	9.5	134	1.4
82	6.7	108	2.8	135	2.6
83	11.9	109	9.5	136	1.1
84	6.7	110	2.1	137	2.1
85	23.8	111	5.6	138	1.2
86	1.9	112	0.7	139	1.0
87	0.6	113	2.3	140	0.5
88	0.4	114	0.6	141	0.7
89	0.8	115	1.4	142	1.4
90	0.5	116	0.5	143	47.6
91	11.4	117	1.3	144	4.1
92	2.6	119	4.8	145	2.0
93	14.3	120	1.5	146	0.5
94	2.7	121	3.5	147	1.5
95	8.1	122	1.4	148	0.6
96	2.7	123	3.0	149	3.3
97	6.7	124	1.6	150	0.9
98	1.9	125	21.0	151	3.5
99	2.0	126	3.2	152	0.8
100	0.9	127	3.0	153	0.9
101	2.1	128	1.3	154	0.5
102	0.5	129	1.2	155	1.0
103	1.0	130	0.5	156	0.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
157	0.5	180	0.2	204	0.9
158	0.3	181	0.2	205	0.5
159	1.6	182	0.1	206	0.6
160	0.5	183	0.2	207	0.4
161	2.1	184	0.5	208	0.2
162	0.5	186	0.3	209	0.2
163	0.5	187	0.2	211	0.1
164	0.2	188	0.1	214	0.1
165	0.4	189	0.5	215	0.1
166	0.2	190	0.1	216	0.1
167	0.3	191	0.2	217	0.1
168	0.3	192	0.1	218	0.3
169	0.3	193	0.3	219	0.2
170	0.1	194	0.2	220	0.3
171	0.2	195	2.7	221	0.9
172	0.1	196	0.5	222	0.3
173	0.3	197	0.2	223	0.2
174	0.2	198	0.1	224	0.1
175	0.4	199	0.3	236	0.5
176	0.3	200	0.1	237	0.2
177	0.7	201	0.2	238	0.1
178	0.4	202	0.1	239	1.1
179	0.4	203	0.3	240	0.3

Base peak: at m/e 43

Metastable ions: the same as Fraction 26

Discussion and conclusions

The general appearance of the mass spectrum of this fraction was similar to that of Fraction 26 (MS12). The metastable peaks were the same in both.

It may be concluded that Fraction 27 contains as its major component an oxygenated sesquiterpene similar to that in Fraction 26.

Fraction 28

Gas-liquid chromatography: this fraction had a small peak which was not completely resolved from 27, a retention time of 29.3 mins., and represented 3.55% of the essential oil. No other data are available.

Fraction 29

Results

Gas-liquid chromatography: this fraction had a small peak, a retention time of 31.9 mins., and represented 2.17% of the essential oil. The peak was augmented by methyl eugenol.

Mass spectral data: only one scan was run on MS12.

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.8	25	1.4	39	20.8
13	0.8	26	3.8	40	3.4
14	3.1	27	25.4	41	65.4
15	4.6	29	28.5	42	9.2
16	0.8	30	0.4	43	100.0
19	0.4	31	6.2	44	3.4

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
45	6.9	75	1.5	103	2.3
46	0.8	77	9.2	104	0.8
47	0.8	78	1.5	105	10.0
50	1.5	79	15.4	106	2.3
51	3.8	80	3.8	107	10.8
52	3.1	81	30.8	108	6.9
53	16.2	82	12.3	109	13.8
54	4.6	83	13.1	110	4.6
55	43.1	84	4.6	111	13.1
56	7.7	85	35.4	112	3.1
57	47.7	86	3.1	113	2.3
58	3.4	87	0.8	114	0.8
59	4.6	89	0.8	115	3.8
62	0.8	90	0.8	116	2.3
63	1.5	91	12.3	117	3.8
64	0.8	92	3.8	118	2.3
65	3.8	93	22.3	119	7.7
66	3.8	94	6.2	120	3.8
67	23.1	95	11.5	121	6.9
68	10.0	96	3.8	122	3.1
69	28.5	97	6.9	123	3.1
70	3.8	98	3.4	124	10.0
71	13.1	99	3.8	125	10.0
72	3.8	100	1.5	126	3.8
73	3.1	101	2.3	127	3.4
74	0.8	102	0.8	128	2.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
129	2.3	153	1.5	177	2.3
130	1.5	154	1.5	178	0.8
131	3.1	155	3.8	179	1.5
132	1.9	156	0.8	180	0.8
133	3.4	157	2.3	181	0.8
134	3.1	158	1.5	184	0.4
135	4.6	159	3.1	185	0.8
136	3.1	160	3.1	187	1.4
137	3.4	161	4.6	189	1.4
138	0.8	162	1.5	190	0.8
139	1.5	163	1.5	191	0.8
140	1.5	164	0.8	192	0.8
141	3.8	165	1.5	193	0.8
142	1.5	166	0.8	194	0.8
143	6.9	167	0.8	195	1.5
144	2.3	168	0.8	199	0.8
145	4.6	169	0.8	200	0.8
146	3.1	170	0.8	202	1.4
147	3.1	171	0.8	203	0.8
148	3.1	173	0.8	204	2.3
150	1.5	174	0.8	205	2.3
151	2.3	175	2.3	206	0.8
152	1.5	176	0.8	207	0.8

Base peak: at m/e 43

Discussion and conclusions

The retention data originally suggested methyl eugenol,

but this was ruled out by the absence of a distinct peak at m/e 178.

The only conclusion which could be derived from the general appearance of the mass spectrum is that Fraction 29 may contain an oxygenated sesquiterpene similar to those in Fractions 26 and 27.

Fraction 32

Gas-liquid chromatography: this fraction had a small broad peak, a retention time of 36.8 mins., and represented 3.17% of the essential oil.

No other data are available.

Fraction 33

Results

Gas-liquid chromatography: this fraction had a broad peak, a retention time of 39.0 mins., and represented 2.96% of the essential oil. The collected viscid liquid, when tested for purity on Se-30, gave a broad peak. The peak was augmented by eugenol.

Mass spectral data: three scans were run on MS12 and four scans on MS9.

Data from MS12:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	15	5.7	27	15.7
13	0.3	25	0.3	29	18.7
14	1.7	26	2.0	30	0.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
31	3.0	64	0.3	90	0.3
38	0.3	65	2.7	91	5.7
39	13.0	66	2.7	92	1.3
40	3.0	67	15.3	93	9.0
41	45.0	68	11.7	94	5.7
42	12.3	69	17.7	95	8.7
43	100.0	70	8.3	96	5.3
44	11.7	71	7.3	97	23.0
45	3.7	72	1.1	98	5.0
46	0.3	73	2.7	99	1.7
47	0.3	74	0.3	100	1.0
48	0.2	75	0.7	101	1.0
50	0.7	76	0.3	102	0.3
51	1.3	77	4.3	103	0.7
52	1.0	78	0.7	104	1.0
53	6.7	79	9.3	105	5.3
54	10.7	80	4.3	106	1.0
55	52.0	81	15.0	107	4.3
56	12.0	82	4.0	108	2.0
57	11.7	83	9.7	109	3.7
58	5.0	84	8.0	110	1.7
59	2.0	85	3.3	111	3.0
60	0.7	86	0.7	112	4.0
61	14.3	87	0.3	113	1.0
62	0.3	88	0.2	114	0.7
63	1.0	89	0.7	115	2.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
116	0.7	142	0.7	169	0.7
117	2.0	143	1.7	171	0.3
118	0.7	144	0.7	172	0.7
119	2.3	145	2.3	173	0.3
120	1.3	146	0.7	174	0.2
121	4.3	147	1.3	175	0.7
122	2.0	148	0.7	176	0.3
123	1.0	150	0.3	177	0.7
124	0.7	151	1.3	178	0.7
125	1.7	152	1.7	179	0.7
126	0.7	153	2.0	181	0.7
127	1.7	154	0.7	182	0.3
128	1.3	155	1.3	183	7.3
129	1.3	156	0.5	184	1.3
130	0.3	157	2.3	185	0.7
131	2.0	158	0.7	187	0.7
132	1.0	159	2.0	189	1.0
133	2.3	160	1.0	191	0.3
134	2.0	161	6.0	195	0.3
135	2.0	162	1.3	196	0.2
136	1.0	163	0.7	197	0.2
137	1.3	164	1.7	198	3.3
138	0.3	165	2.0	199	0.7
139	0.7	166	0.7	200	0.3
140	1.0	167	1.7	201	0.3
141	1.3	168	2.0	202	0.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
203	0.7	205	1.3	207	0.5
204	2.7	206	0.7	208	0.2
				209	0.2

Base peak: at m/e 43

Metastable ions:

Diffuse peaks	Possible break-down reactions
31.1	$302^+ \longrightarrow 97^+ + 205$
164.5	$238^+ \longrightarrow 198^+ + 40$
45.5	$198^+ \longrightarrow 95^+ + 103$
154.5	$183^+ \longrightarrow 168^+ + 15$
133.1	$167^+ \longrightarrow 149^+ + 18$
68.5	$161^+ \longrightarrow 56^+ + 105$
54.7	$82^+ \longrightarrow 67^+ + 15$
24.4	$69^+ \longrightarrow 43^+ + 26$
25.2	$67^+ \longrightarrow 41^+ + 26$

Data from MS9:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	24	0.2	33	0.3
13	1.0	25	1.3	39	0.7
14	3.3	26	6.5	40	0.3
15	7.2	27	48.3	41	2.7
16	0.4	28	16.7	42	4.0
17	4.2	29	31.7	43	38.3
18	38.3	30	6.7	44	1.8
19	4.3	31	100.0	45	95.0
20	0.1	32	1.7	46	61.7

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
47	1.0	78	0.1	108	0.3
48	0.1	79	0.7	109	0.8
51	0.1	80	0.4	110	0.2
52	0.1	81	1.3	111	0.5
53	0.4	82	0.5	112	0.4
54	0.8	83	0.8	113	0.1
55	3.8	84	0.6	115	0.1
56	1.0	85	0.3	117	0.1
57	0.7	86	0.1	118	0.1
58	0.4	87	0.1	119	0.3
59	0.2	91	0.3	120	0.1
61	1.7	92	0.1	121	0.5
62	0.1	93	0.8	122	0.3
63	0.1	94	0.6	123	0.2
65	0.1	95	0.8	124	0.1
66	0.2	96	0.5	125	0.2
67	1.5	97	2.7	126	0.1
68	1.3	98	0.5	127	0.1
69	1.7	99	0.3	128	0.1
70	0.6	100	0.1	129	0.1
71	0.9	101	0.1	131	0.1
72	0.2	103	0.1	132	0.1
73	0.2	104	0.1	133	0.1
74	0.1	105	0.4	134	0.1
76	0.1	106	0.1	135	0.2
77	0.3	107	0.4	136	0.1

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
137	0.2	153	0.1	169	0.1
138	0.2	154	0.1	172	0.1
139	0.1	155	0.1	175	0.1
140	0.1	156	0.1	177	0.1
141	0.1	157	0.3	179	0.1
142	0.1	158	0.1	183	0.8
143	0.4	159	0.2	184	0.1
144	0.1	160	0.1	189	0.1
145	0.2	161	0.5	198	0.4
146	0.1	162	0.1	199	0.1
147	0.1	163	0.1	203	0.1
148	0.1	164	0.2	204	0.3
149	0.1	165	0.1	205	0.1
150	0.1	166	0.1	206	0.1
151	0.1	167	0.1	220	0.1
152	0.2	168	0.2	222	0.1

Base peak: at m/e 31

Data from MS9: direct probe, ion source temperature 100°C:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.5	19	1.7	30	1.1
13	4.8	26	2.6	31	9.3
14	0.9	27	14.0	32	2.5
15	4.8	28	20.2	34	0.3
16	1.6	28	6.2	35	15.5
17	21.7	29	12.4	36	14.0
18	94.6	29	4.7	37	4.8

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
38	4.3	63	0.9	85	9.3
39	12.4	64	0.5	86	1.4
40	3.1	65	2.8	86	1.1
41	31.0	66	1.9	87	9.2
42	5.9	67	15.5	87	1.2
43	58.9	68	12.4	88	0.9
43	9.3	69	14.0	89	1.1
44	6.1	70	3.9	90	0.3
45	7.4	71	15.5	91	5.1
46	1.9	72	2.6	92	1.9
47	35.7	73	3.7	93	6.7
48	15.5	74	1.2	94	4.2
49	12.4	75	1.2	95	7.8
50	4.5	76	1.1	96	3.3
51	2.7	77	4.5	97	4.5
52	1.4	78	1.4	98	2.3
53	9.3	79	5.6	99	6.4
54	6.2	80	2.3	100	2.2
55	20.2	81	14.0	101	2.3
56	5.0	82	8.8	102	0.9
57	5.0	82	3.9	103	0.9
58	3.4	83	100.0	104	0.6
59	5.6	83	9.3	105	3.3
60	1.2	84	4.0	106	1.2
61	3.6	84	3.6	107	4.3
62	0.6	85	65.1	108	3.0

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
109	8.1	129	1.4	155	1.2
110	4.0	130	0.5	156	1.2
111	7.1	131	0.9	157	0.8
112	2.6	132	0.8	158	0.3
113	1.9	133	1.6	159	1.6
114	0.9	134	1.2	160	1.2
115	1.7	135	2.3	161	1.9
116	1.1	136	1.2	162	1.1
117	1.4	137	2.2	163	1.1
117	1.1	138	2.2	164	1.2
118	3.3	139	2.0	165	2.3
118	0.8	140	0.9	166	1.1
119	2.5	141	1.9	167	1.9
119	1.4	142	2.3	168	3.0
120	3.3	143	18.6	169	1.2
120	1.4	144	2.3	170	1.9
121	3.3	145	1.4	171	1.9
121	0.5	146	0.8	172	0.3
122	1.9	147	1.1	173	0.8
122	1.4	148	0.8	174	0.3
123	2.6	149	1.6	175	0.9
124	1.2	150	1.6	176	0.6
125	7.1	151	2.0	177	1.4
126	2.6	152	5.3	178	0.9
127	2.5	153	3.1	179	0.9
128	1.9	154	1.1	180	0.5

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
181	1.1	205	1.2	229	0.1
182	0.9	206	0.3	230	0.1
183	15.5	207	0.3	231	0.1
184	2.5	208	0.3	232	0.1
185	0.8	209	0.3	233	0.1
186	0.3	210	0.3	234	0.1
187	0.3	211	0.3	235	0.1
188	0.3	212	0.3	236	0.6
189	0.5	213	0.1	237	0.3
190	0.3	214	0.1	238	0.9
191	0.3	215	0.1	239	0.9
192	0.5	216	0.1	240	0.3
193	0.9	217	0.2	241	0.1
194	1.1	218	0.3	242	0.1
195	2.3	219	0.1	244	0.1
196	1.1	220	1.1	245	0.1
197	0.8	221	0.8	246	0.1
198	9.3	222	0.6	251	0.1
199	1.4	223	0.9	252	0.1
200	0.3	224	0.6	253	0.1
201	0.3	225	0.3	254	0.2
202	0.3	226	0.3	255	0.1
203	0.5	227	0.3	256	0.2
204	1.1	228	0.2		

Base peak: at m/e 83

Data from MS9: direct probe, ion source temperature 50°C:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
15	2.3	48	0.5	73	7.8
17	23.4	49	0.4	74	1.7
18	100.0	50	1.3	75	1.3
19	0.7	51	1.8	76	1.8
20	0.3	52	1.0	77	5.0
26	1.8	53	5.7	78	1.6
27	10.4	54	6.5	79	7.8
28	18.2	55	29.9	80	3.0
28	3.9	56	10.4	81	18.2
29	13.0	57	10.4	82	15.6
29	3.9	58	6.5	83	13.0
31	7.8	59	6.5	83	5.2
32	2.9	60	5.2	84	9.1
35	0.5	61	6.5	85	13.0
36	1.0	62	0.8	85	3.2
39	13.0	63	1.0	86	2.1
40	2.6	64	0.5	87	2.6
41	31.2	65	5.2	88	1.6
42	6.8	66	2.2	89	1.6
43	59.8	67	18.2	90	0.8
43	15.6	68	16.9	91	14.3
44	6.0	69	20.8	92	2.6
45	6.2	70	7.8	93	10.4
46	1.3	71	20.8	94	9.1
47	1.0	72	3.9	95	15.6

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
96	7.8	122	2.1	148	1.6
97	13.0	123	5.7	149	13.0
98	6.5	124	3.1	150	3.1
99	10.4	125	13.0	151	3.9
100	2.9	126	3.6	152	7.8
101	3.4	127	4.4	153	5.2
102	1.3	128	2.9	154	2.3
103	1.3	129	2.3	155	3.6
104	1.8	130	1.3	156	2.3
105	5.2	131	1.8	157	1.6
106	2.1	132	1.8	158	1.0
107	7.8	133	3.4	159	4.4
108	5.2	134	2.1	160	1.8
109	13.0	135	4.7	161	2.9
110	10.4	136	2.6	162	2.1
111	13.0	137	4.7	163	2.3
112	6.5	138	5.2	164	2.1
113	3.4	139	3.9	165	2.9
114	2.3	140	2.3	166	1.6
115	2.9	141	2.9	167	2.9
116	1.6	142	3.4	168	3.1
117	1.8	143	20.8	169	1.6
118	1.3	144	3.1	170	1.0
119	4.4	145	2.3	171	1.3
120	2.3	146	1.0	172	0.8
121	2.9	147	2.3	173	1.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
174	1.0	200	0.7	226	0.8
175	1.8	201	0.7	227	0.5
176	1.6	202	1.0	228	0.3
177	3.6	203	1.6	229	0.3
178	2.1	204	1.6	230	0.3
179	1.8	205	2.1	231	0.3
180	1.6	206	2.6	232	0.4
181	1.8	207	1.3	233	0.3
182	1.3	208	0.8	234	0.7
183	13.0	209	0.8	235	0.4
184	2.6	210	0.8	236	1.6
185	1.3	211	0.9	237	0.8
186	0.8	212	1.0	238	2.5
187	1.0	213	0.5	239	1.7
188	0.8	214	0.8	240	0.6
189	1.0	215	0.5	241	0.6
190	0.7	216	0.5	242	0.3
191	1.3	217	0.4	243	0.3
192	1.0	218	0.8	244	0.2
193	1.8	219	0.8	245	0.2
194	1.8	220	2.6	246	0.2
195	3.1	221	2.1	247	0.2
196	1.6	222	1.3	248	0.2
197	1.6	223	1.8	249	0.1
198	7.8	224	1.0	250	0.2
199	2.1	225	0.8	251	0.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
252	0.6	274	0.2	296	0.2
253	0.5	275	0.2	297	0.1
254	0.8	276	0.2	298	0.2
255	0.4	277	0.1	299	0.1
256	1.6	278	0.2	300	0.2
257	0.4	279	0.6	301	0.1
258	0.6	280	0.2	302	0.2
259	0.5	281	0.3	303	0.1
260	0.2	282	0.2	304	0.2
261	0.3	283	0.2	305	0.2
262	0.3	284	0.2	306	0.2
263	0.2	285	0.1	307	0.1
264	0.2	286	0.2	308	0.1
265	0.1	287	0.2	309	0.1
266	0.2	288	0.2	310	0.1
267	0.2	289	0.2	311	0.1
268	0.2	290	0.2	312	0.6
269	0.2	291	0.1	313	0.2
270	0.2	292	0.7	314	0.1
271	0.1	293	0.1	315	0.1
272	0.2	294	0.2	316	0.2
273	0.1	295	0.1	317	0.1

Base peak: at m/e 18

Data from MS9: direct probe, ion source temperature 100°C:

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
12	0.3	39	20.4	63	2.2
13	0.3	40	5.1	64	0.9
14	0.9	41	74.8	65	13.6
15	6.8	42	17.9	66	3.4
17	6.8	43	96.6	67	23.0
18	22.1	43	45.9	68	17.9
19	1.2	44	10.2	69	35.7
24	0.2	45	13.6	70	23.8
25	0.4	46	1.7	71	28.9
26	2.5	47	0.3	72	5.1
27	22.1	48	0.2	73	6.8
28	6.8	49	0.3	74	2.4
28	7.7	50	3.4	75	2.6
29	36.6	51	4.1	76	9.4
29	6.0	52	1.7	77	11.1
30	2.2	53	10.2	78	2.6
30	0.6	54	11.1	79	11.1
31	11.1	55	62.1	80	5.1
32	0.9	56	22.1	81	20.4
32	0.7	57	47.6	82	15.3
33	0.1	58	7.7	83	19.6
35	0.2	59	7.7	84	11.1
36	0.7	60	4.3	85	11.9
37	0.5	61	6.8	86	2.4
38	1.2	62	1.0	87	2.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
88	1.2	114	2.6	140	2.2
89	2.2	115	3.9	141	3.1
90	1.4	116	1.5	142	2.0
91	37.4	117	1.9	143	10.2
92	6.0	118	1.2	144	2.2
93	12.8	119	5.1	145	2.6
94	6.8	120	1.7	146	1.2
95	13.6	121	6.0	148	2.4
96	7.7	122	6.0	149	100.0
97	14.5	123	9.4	150	14.5
98	6.8	124	4.3	151	5.1
99	8.5	125	9.4	152	3.4
100	5.1	126	5.1	153	4.3
101	5.1	127	5.6	154	1.9
102	1.5	128	4.3	155	3.2
103	1.5	129	4.3	156	1.7
104	12.8	130	1.3	157	7.7
105	10.2	131	2.2	158	2.0
106	2.4	132	8.5	159	3.6
107	9.4	133	3.4	160	1.5
108	5.1	134	1.5	161	2.6
109	12.8	135	6.8	162	1.7
110	11.1	136	2.4	163	2.0
111	11.1	137	5.1	164	1.2
112	12.8	138	6.8	165	2.6
113	12.8	139	7.7	166	1.2

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
167	28.9	193	1.5	219	1.0
168	2.9	194	1.5	220	1.7
169	1.5	195	1.7	221	1.2
170	0.9	196	1.2	222	0.5
171	2.7	197	2.0	223	0.7
172	1.0	198	1.2	224	0.5
173	1.5	199	7.7	225	0.7
174	1.0	200	2.0	226	0.7
175	2.4	201	3.7	227	1.4
176	1.4	202	1.5	228	1.2
177	2.6	203	2.2	229	0.7
178	2.3	204	0.8	230	0.7
179	1.4	205	2.6	231	0.5
180	0.9	206	11.1	232	0.3
181	1.4	207	2.0	233	0.3
182	0.9	208	0.7	234	0.5
183	1.7	209	1.0	235	0.5
184	0.9	210	0.5	236	1.0
185	2.9	211	1.0	237	0.9
186	1.2	212	0.7	238	2.0
187	2.2	213	1.5	239	1.0
188	1.4	214	1.2	240	0.5
189	1.5	215	0.9	241	0.5
190	0.8	216	0.8	242	0.3
191	1.2	217	1.5	243	0.5
192	0.9	218	0.9	244	0.3

m/e	rel. abundance	m/e	rel. abundance	m/e	rel. abundance
245	0.3	268	0.2	291	0.1
246	0.2	269	0.3	292	0.1
247	0.3	270	0.3	293	0.1
248	0.2	271	0.3	294	0.1
249	0.3	272	0.3	295	0.2
250	0.3	273	0.2	296	0.1
251	0.2	274	0.2	297	0.3
252	0.3	275	0.2	298	0.1
253	0.7	276	0.2	299	1.0
254	0.7	277	0.2	300	0.3
255	0.5	278	0.2	301	0.2
256	1.0	279	19.6	302	0.1
257	0.5	280	4.3	303	0.1
258	0.4	281	0.5	304	0.1
259	0.5	282	0.3	305	0.1
260	0.2	283	0.5	306	0.1
261	0.7	284	0.3	307	0.1
262	0.5	285	0.3	308	0.1
263	0.3	286	0.1	309	0.1
264	0.2	287	0.1	310	0.1
265	0.3	288	0.1	311	0.1
266	0.3	289	0.1	312	2.6
267	0.3	290	0.1	313	0.7
				314	0.2

Base peak: at m/e 149

An accurate mass determination of the following ions was

made:

m/e 279: measured 279.1601; calculated for $C_{16}H_{23}O_4$: 279.1596.

m/e 204: measured 204.1875; calculated for $C_{15}H_{24}$: 204.1878.

m/e 198: measured 198.1406; calculated for $C_{15}H_{18}$: 198.1409.

m/e 183: measured 183.1178; calculated for $C_{14}H_{15}$: 183.1174.

m/e	Elemental composition
279	$C_{16}H_{23}O_4$
204	$C_{15}H_{24}$
198	$C_{15}H_{18}$
183	$C_{14}H_{15}$
312	$C_{19}H_{20}O_4$

Ultraviolet data: 2 mg/100 ml

λ_{max}^{EtOH}	$E_{1\ cm}^{1\%}$
229	600
290	70
305	10

Infrared data:

3480 (m, broad), 2920 (s, broad), 2850 (s), 2710 (w), 1730 (s, broad), 1455 (m), 1380 (sh), 1365 (m), 1235 (s, broad), 1110 (w), 1085 (w), 1060 (m), 1030 (m, broad), 980 (sh), 905 (w) cm^{-1}.

An infrared spectrum of an authentic sample of eugenol, $C_{10}H_{12}O_2$, was recorded:

3500 (s, broad), 3070 (m), 3000 (m), 2970 (m), 2930 (m), 2900 (m), 2840 (m), 1850 (w, broad), 1640 (m), 1610 (s), 1510 (s), 1455 (s), 1430 (s), 1360 (s),1260 (s), 1225 (s), 1200 (s), 1145 (s), 1115 (s), 1025 (s), 985 (m), 940 (sh),

900 (s), 840 (m), 810 (s), 785 (s), 735 (s), 710 (sh) cm^{-1}. Nuclear magnetic resonance data: figure 5, sweep width 500 cps, sweep offset 150 cps.

Discussion and conclusions

The high-resolution mass spectrometry established the formulas: $C_{15}H_{24}$, $C_{15}H_{18}$ and $C_{14}H_{15}$ for m/e 204, 198 and 183.

The presence of oxygen was obvious from the base peak (m/e 31) and m/e 45, the carbonyl absorption in the infrared spectrum, and the NMR data. This, together with the peaks below m/e 204, which could not be derived from it, indicated that the molecular ion was missing.

The general appearance of both mass spectra recorded on MS9 and MS12 looked similar, and indicated the presence of alkyl hydrocarbon moiety. The number of carbon atoms could be traced up to C_9. The peak at m/e 61 suggested an acetate ester (130). The low mass end of the spectra looked different. The base peak in the mass spectrum recorded on MS9 was at m/e 31, while the second and third most intense peaks at m/e 45 and 46. The base peak in the mass spectrum recorded on MS12 was at m/e 43, peaks at m/e 31 and 45 were small, and m/e 46 was missing.

Attempts were made to avoid decomposition in the inlet system. The sample was introduced by direct probe. Three scans were recorded, two at 100°C and one at 50°C. The mass spectra were extended at the high mass end. There were several even-mass ions: m/e 206, 212, 220, 236, 238

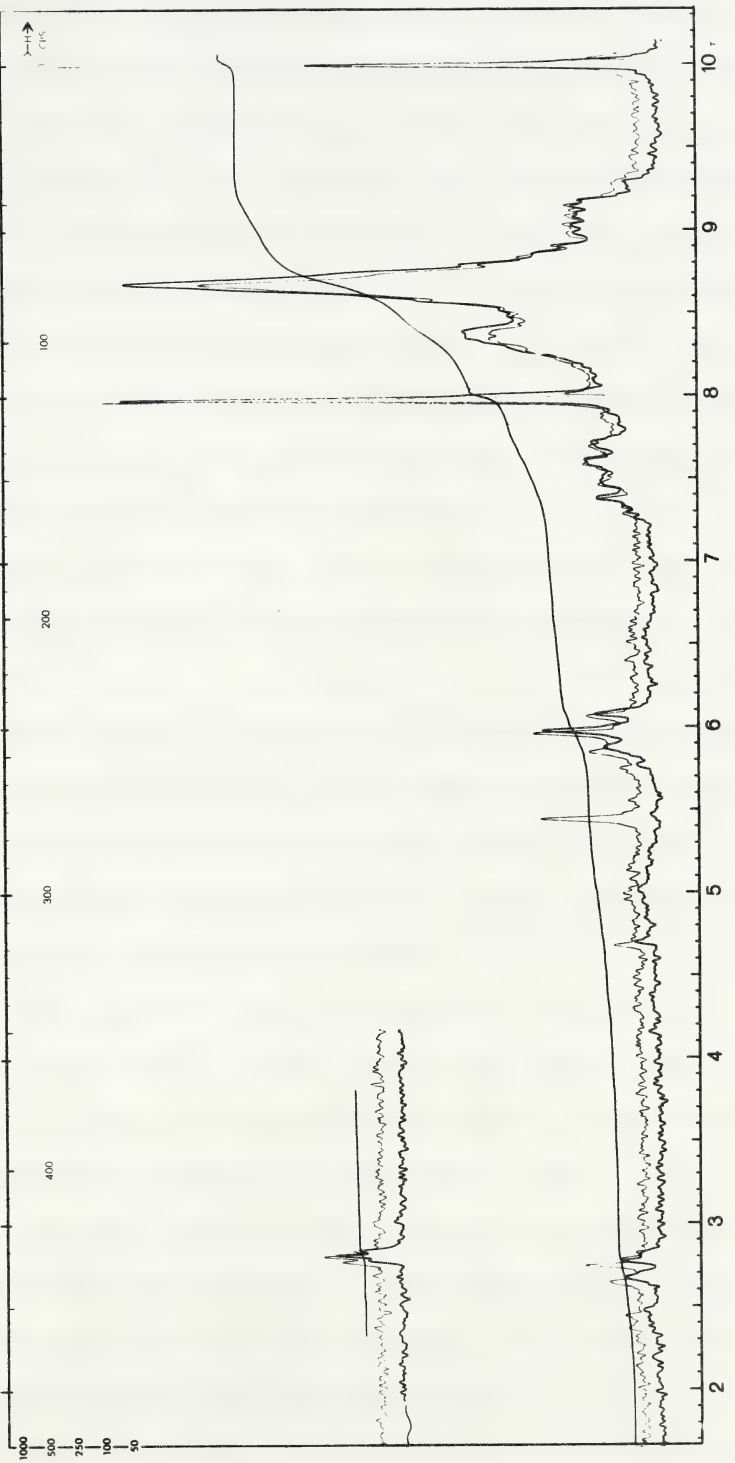


Figure 5. NMR Spectrum of Fraction 33

and 256. One of the spectra (recorded at 50°C) showed small peaks at m/e 312 and 279, and a distinct peak at m/e 149. In another spectrum (recorded at 100°C) the peak at m/e 312 was more distinct, 279 more intense, and 149 was the base peak. In the same spectrum there was a distinct peak at m/e 280, and a large one at m/e 167. A suitable metastable indicated the direct transition: $167^{+} \longrightarrow 149^{+} + 18$. The high-resolution mass spectrometry established the formulas $C_{19}H_{20}O_4$ and $C_{16}H_{23}O_4$ for m/e 312 and 279. The accurate mass of m/e 256 could not be determined.

Obviously m/e 279 can not be derived from m/e 312 indicating the presence of more than one component in this fraction.

The three spectra obtained by direct probe, although they showed some differences, had common features with the spectrum recorded on MS12. The peaks at m/e 206, 204, 198 and 183 were present in all spectra. At the low mass end they had the same general appearance.

The base peak at m/e 149 suggested long chain phthalate esters (254). These esters are widely used in oil diffusion pumps. The presence of strong peak at m/e 149 indicates most probably contamination (254). It was understood that the diffusion oil used in the department of mass spectrometry contained no phthalate esters. So it may be contamination from other source. The large peak at m/e 279 may belong to the phthalate ester.

The peaks at higher mass than 256 were assumed to

be due to contamination. An attempt was made to correlate m/e 204 ($C_{15}H_{24}$), 198 ($C_{15}H_{18}$), and m/e 183 ($C_{14}H_{15}$) to each other and to higher mass ions.

The appearance of small peaks one mass unit apart between m/e 204 and 198, in the mass spectrum recorded on MS12, may be indicative of degradation (255). This sequence has an analogy in the chemistry of cadinenes. Cadinene ($C_{15}H_{24}$) could be dehydrogenated to cadalene ($C_{15}H_{18}$) (240). The fragment ion (m/e 183) may be derived from m/e 198 by the expulsion of a methyl radical. It was not possible to correlate m/e 204 to any of the higher even masses.

A suitable metastable indicated the transition: $238^{+} \longrightarrow 198^{+} + 40$. The formula of m/e 238 may be deduced as $C_{18}H_{22}$ which can not be the molecular ion because of the lack of oxygen. If m/e 238 was assumed to be derived from m/e 256 by the loss of water, the formula $C_{18}H_{24}O$ can not also be qualified as the molecular ion, which is expected to contain more than two oxygen atoms (from IR and NMR data, discussed later).

The molecular ion of the major component of Fraction 33 could not be assigned, but some structural information was derived from the collected data.

The infrared and NMR spectra excluded aromaticity.

The ultraviolet data indicated the presence of unsaturation and conjugation. The weak C-H stretching and bending at 3070 and 905 cm^{-1} in the infrared spectrum, and the absorption at τ 2.6-2.8 in the NMR spectrum indicated that

the chromophore was not completely substituted. The NMR signal suggested protons such as: $\overset{H}{}\!\!>C=C<$ conjugated, or $\underline{H}>C=C<^{H}_{OC(=O)OR}$.

The strong broad carbonyl absorption band could fit saturated aliphatic aldehydes and saturated esters. The strong broad absorption at 1235 cm^{-1} suggested acetate. The two absorption bands at 2710 and 2850 cm^{-1} suggested aliphatic aldehydes.

The one-proton triplet at τ0.3 in the NMR confirmed the presence of aldehyde. The absorption at τ7.6 may account for the CH_2 next to the aldehyde group, which is split into a triplet by an adjacent CH_2 and again into a doublet by the aldehydic proton. The sharp singlet at τ8 accounted for the methyl group of acetate $CH_3C(=O)O$. The triplet at τ5.9, which is suggested to be due to CH_2-O-CO, may belong to the acetate. The absorption at τ8.4 and τ8.6-8.7 may contain protons such as $CH_3C{=}C$, $C\text{-}CH_2\text{-}C\text{-}C{=}C$, and $C\text{-}CH_2\text{-}C$, $CH_2\text{-}C\text{-}C{=}CR_2$, respectively.

The distinct peak at m/e 61 in all the mass spectra of Fraction 33 confirmed the existence of an acetate ester. The large peak at m/e 43, which has been shown by high-resolution to be largely due to CH_3CO^+, was in favor of acetate and aldehyde.

In conclusion, Fraction 33 seems to be a mixture of more than one component. Although detection of the molecular ion of the major component was not possible, some structural features could be deduced from the infrared, ultraviolet, NMR and mass spectra. Fraction 33 contains a component or

components which are: aliphatic, substituted conjugated diene, acetate ester, and saturated aldehyde.

The Mass Spectra of Oplopanone

The mass spectrum of oplopanone recorded on MS9 (Figure 2) showed at the high mass end a large peak at m/e 238 corresponding to a stable molecular ion. The distinct peak at m/e 220 resulted from the loss of water from the molecular ion. The peaks at m/e 205 and 177 correspond to the loss of a methyl and probably isopropyl radical from m/e 220.

At the low mass end the general appearance of clusters of peaks 14 mass units apart indicated the alkyl hydrocarbon nature, while the peaks at m/e 79, 91 and 105 indicated the formation of aromatic rings during fragmentation. The base peak at m/e 43 was shown by the high-resolution to be split. The more intense peak at lower mass indicated that the higher portion of charge was carried by CH_3CO+ (129).

A fragmentation pathway is postulated for oplopanone which could account for the intense peaks in the spectrum. This is in agreement that the simple rules of fragmentation in mass spectrometry may be applied to large and complicated molecules of nature.

The mass spectrum of oplopanone recorded on MS12 (Figure 3) was different in many respects from that recorded on MS9 indicating change in structure after passing in the Lac-728 column. Decomposition was apparent from the

presence of successive peaks one mass-unit apart which indicates degradation.

At the high mass end of the spectrum the peak corresponding to the molecular ion was missing. The highest mass ion was m/e 227. The peak at m/e 220 ($M-H_2O$) was large. The peak at m/e 205 was strikingly intense, while that at m/e 177 was about the same intensity as in the mass spectrum recorded on MS9. The third most intense peak was at m/e 145, while the two peaks at m/e 153 and 135, which were intense and characteristic in the mass spectrum recorded on MS9, were negligible. The base peak was at m/e 18.

Comparison of the mass spectra of oplopanone recorded on both MS9 and MS12 with those of the components of the volatile oil of Oplopanax horridum indicated the absence of oplopanone in the oil prepared by steam distillation.

The Postulated Fragmentation Pathway of Oplopanone

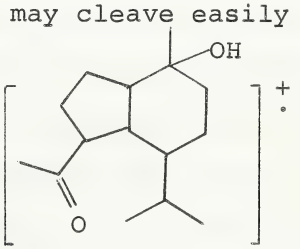
Diagrams used in this postulated fragmentation pathway are only to account for the electrons. They are not meant to represent the actual structure of the ions, as a detailed mechanism for the formation of such peaks can only be given by the study of isotopically enriched materials.

The attached groups to the rings may cleave easily yielding the following fragment ions:

m/e 223 $\xleftarrow{-CH_3\cdot}$

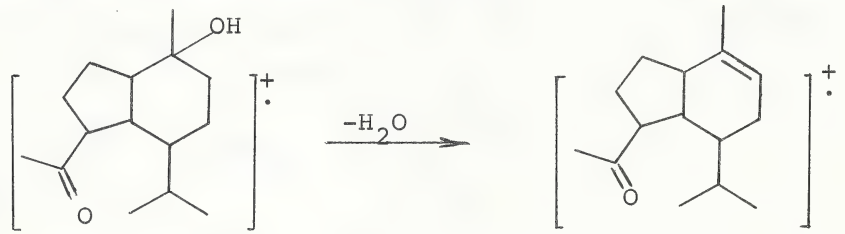
m/e 221 $\xleftarrow{-OH\cdot}$

m/e 195 $\xleftarrow{-C_3H_7\cdot}$



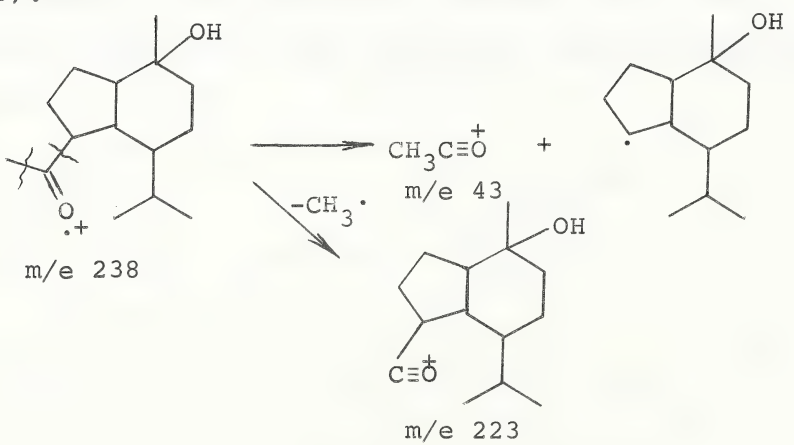
m/e 238 $[M]^{+\cdot}$

As an alcohol oplopanone eliminates a water molecule to give m/e 220. Elimination of water is easier from primary than tertiary alcohols, but it is possible that thermal elimination of water may arise in the inlet system. In this case loss of water from tertiary alcohols is easier (199, 144). The fragment ion corresponding to $M-H_2O$ may be represented for convenience as follows:



m/e 238[M]$^{+\cdot}$ m/e 220

As a ketone α-cleavage yields the stable acylium ions (203):

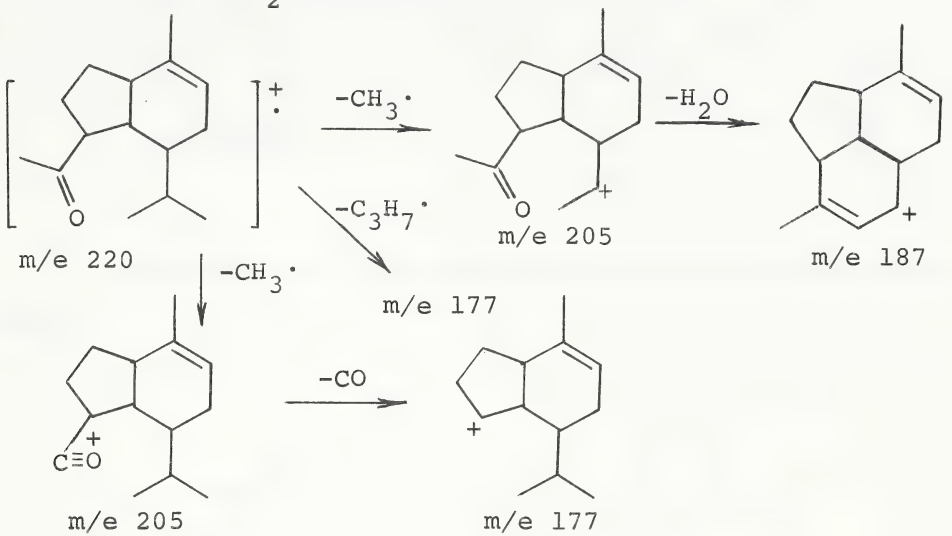


m/e 238 m/e 223

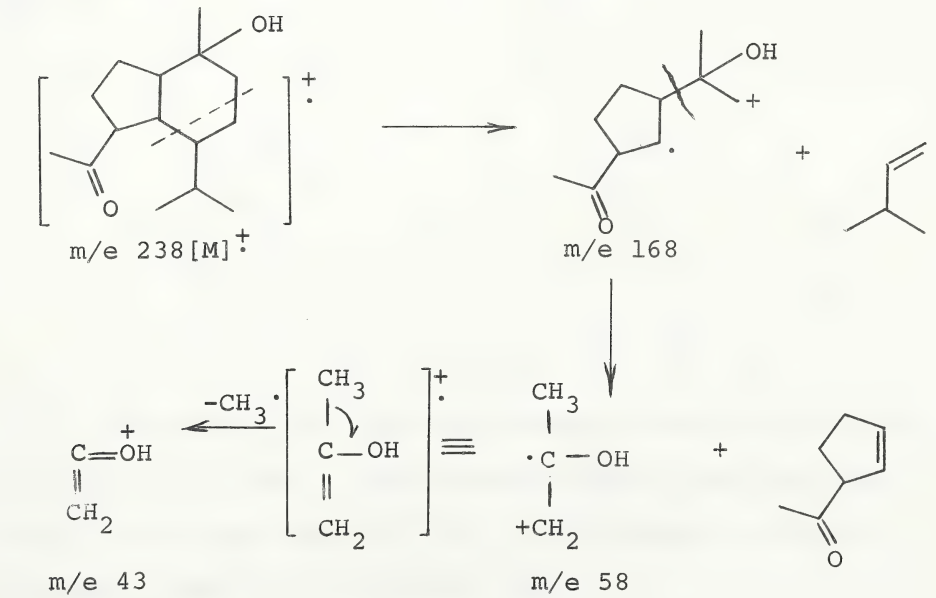
It was noticed that there were two peaks corresponding to the loss of a methyl radical. One is derived directly from the molecular ion and the other from $M-H_2O$ ion. The peak due to $M-CH_3$ was small although theoretically there are three sites for the loss of a methyl radical to yield stable

ions.

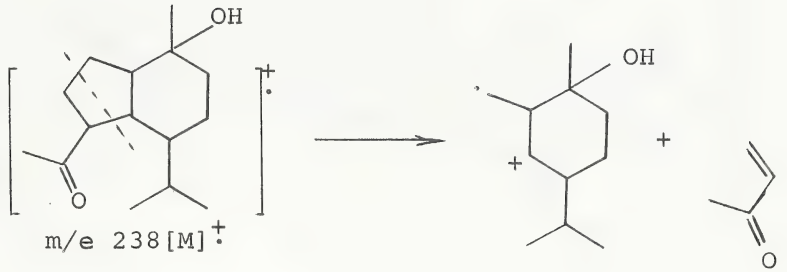
The peaks at m/e 187 and 177 may be accounted for as derived from M-H_2O as follows:



The peak at m/e 168 may be explained as derived from the molecular ion by the release of stable molecules as follows:

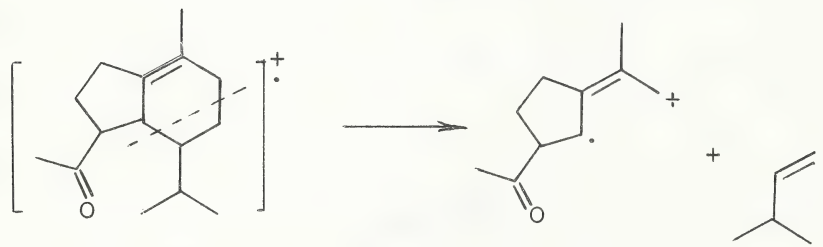


or



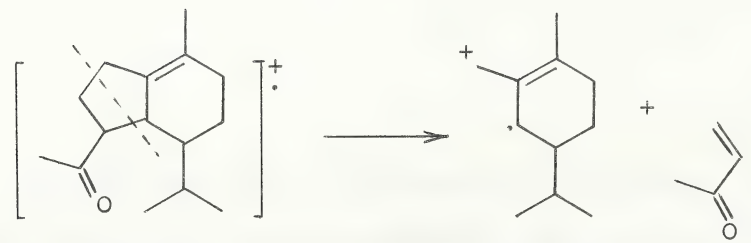
m/e 238[M]$^{+\cdot}$ m/e 168

Similarly the m/e 150 fragment may be derived from $M-H_2O$:



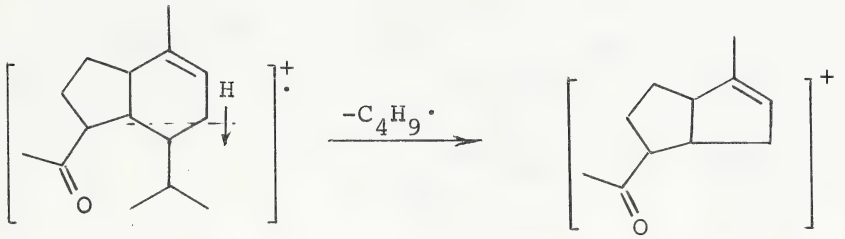
m/e 220 m/e 150

or



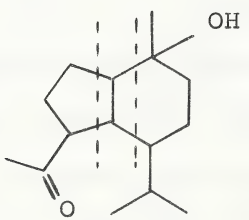
m/e 220 m/e 150

The small but distinct group of peaks at m/e 163, 162, 161, 160 and 159 may be explained as derived from $M-H_2O$ ion by the loss of $C_4H_9\cdot$, and successive loss of hydrogen atoms to form double bonds:

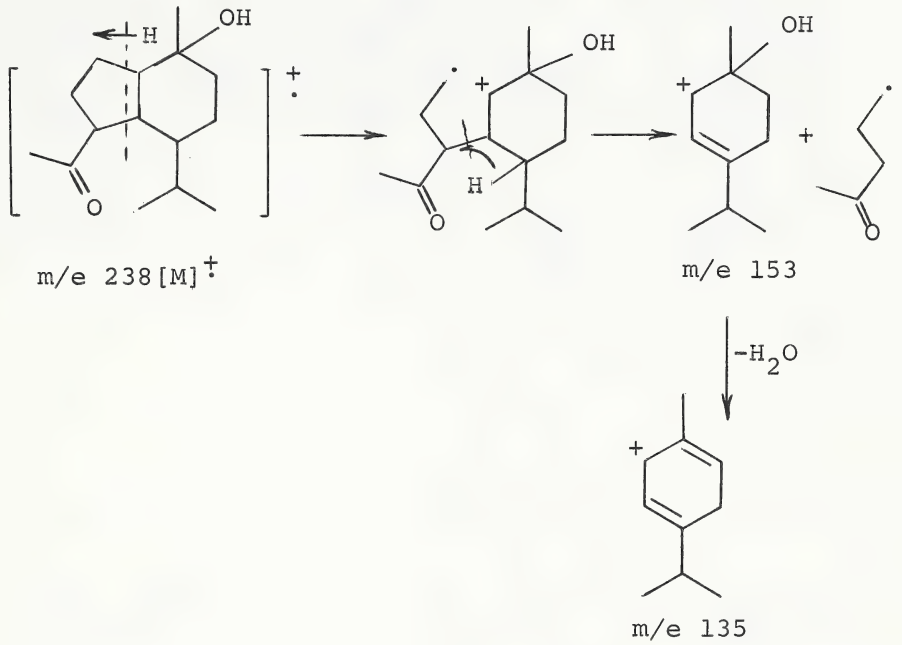


m/e 220 m/e 163

The following series of fragment ions may be accounted for by postulating the fragmentation of the ring system at any pair of the positions shown by dotted lines to give fragment pairs with or without hydrogen transfer (256, 185, 257):

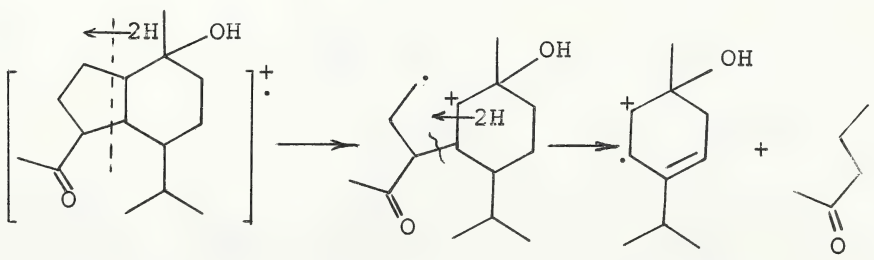


If the two rings of oplopanone fall apart, the charge retained on the six membered ring, and one hydrogen atom transferred from the charge retaining ring, the large peaks at m/e 153 and 135 may be explained as follows:

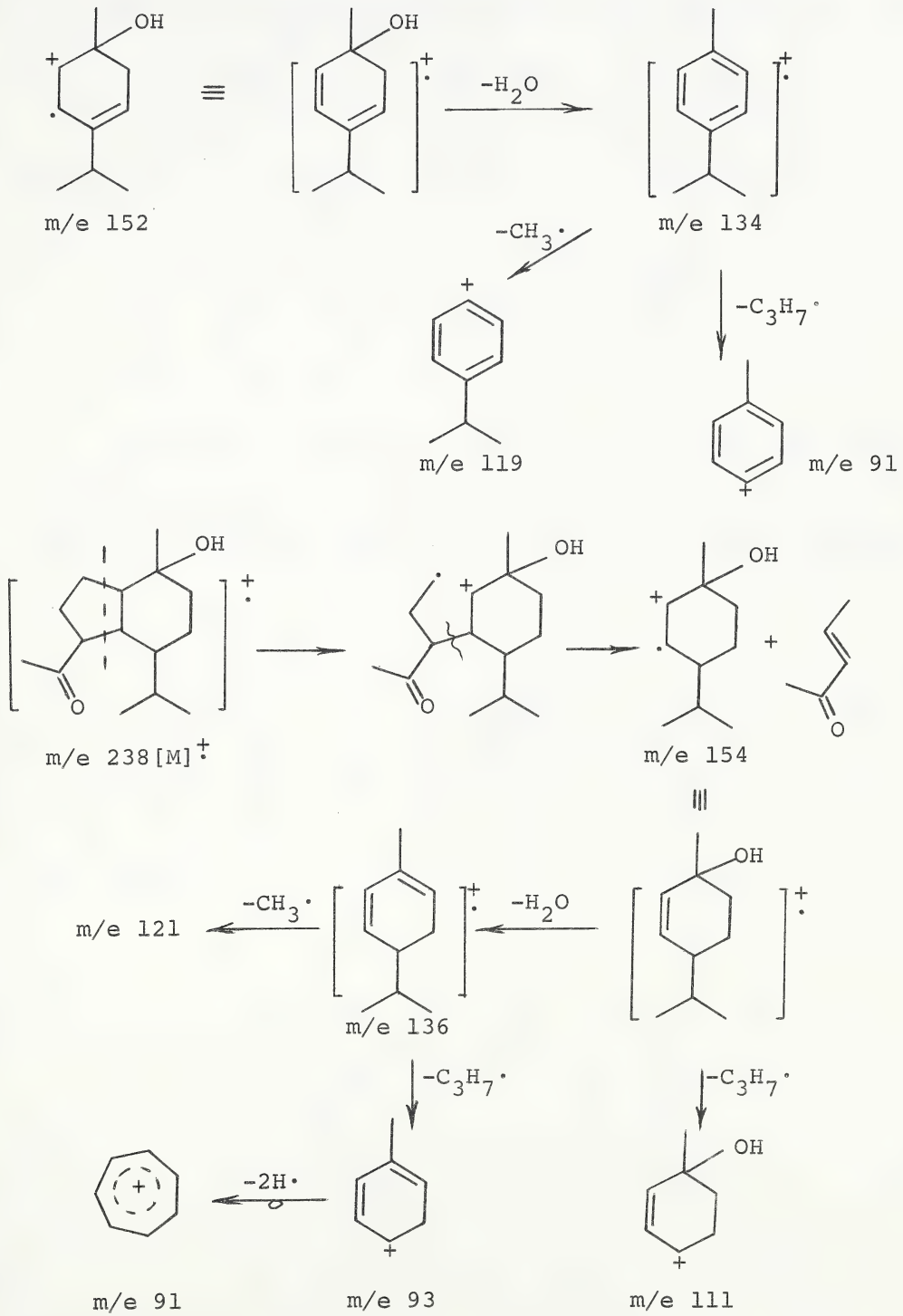


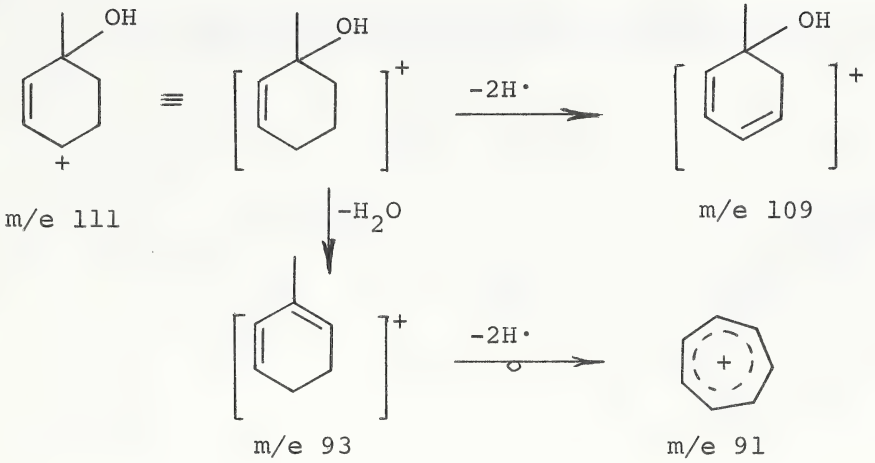
m/e 238[M]$^{+\cdot}$ m/e 153 m/e 135

The previous cleavage, with transfer of two hydrogen atoms, explains the peaks at m/e 152, 134, 119 and 91. If the same cleavage occurs without any hydrogen transfer the peaks at m/e 154, 136, 121, 111, 109, 93 and 91 may be accounted for. This is shown by the following diagrams:

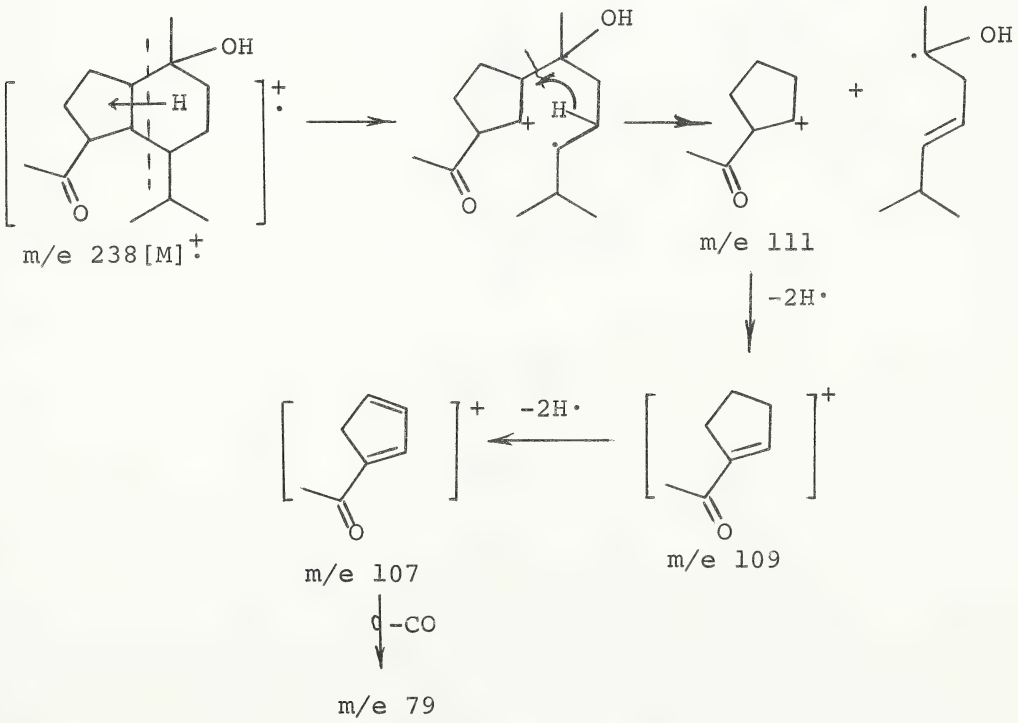


m/e 238 m/e 152

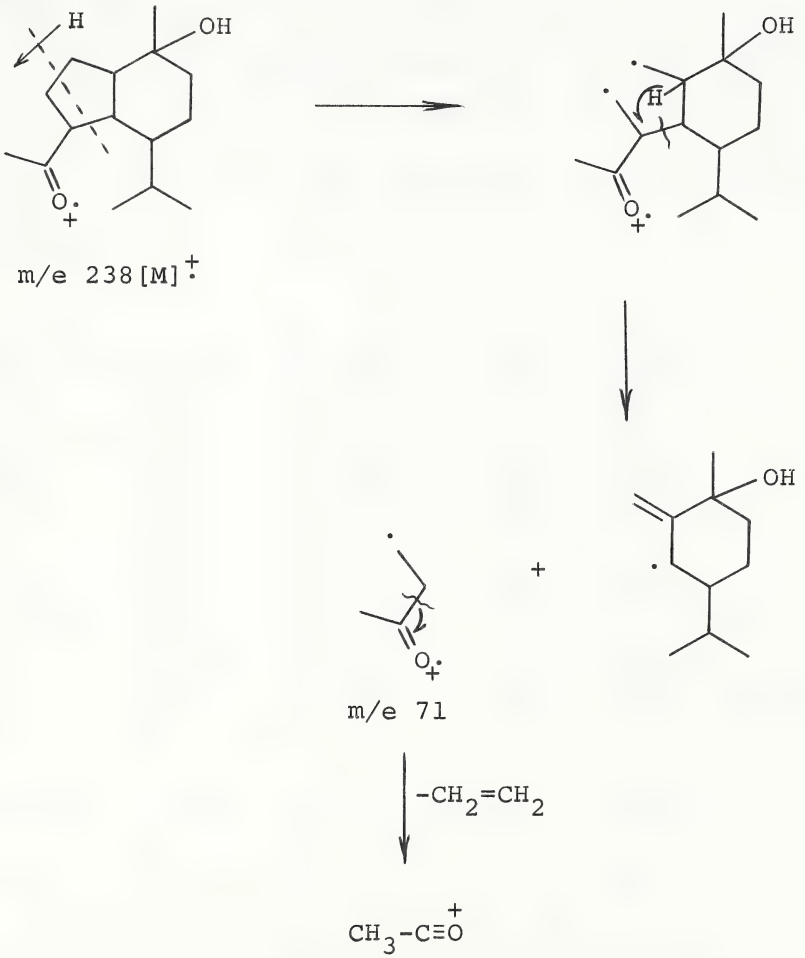

OH
≡
-H2O
m/e 152
m/e 134
-CH3·
-C3H7·
m/e 119
m/e 91
m/e 238[M]+·
m/e 154
m/e 121
m/e 136
m/e 111
m/e 93
m/e 91
-2H·



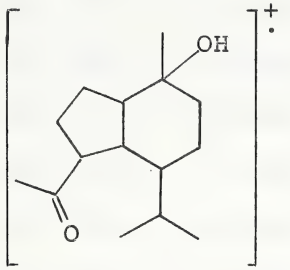
The peaks at m/e 111, 109, 107 and 79 may also be explained by postulating cleavage of rings with positive charge retained on the five membered ring in the following fashion:



The large peak at m/e 71 may be rationalized as follows:



Summary of Oplopanone Fragmentation



→	m/e 223	-15 ($CH_3\cdot$)
→	m/e 195	-43 ($C_3H_7\cdot$)
→	m/e 221	-17 ($HO\cdot$)
→	m/e 220	-18 (H_2O)
→	m/e 43	-195 ($C_{13}H_{23}O\cdot$)

$C_{15}H_{26}O_2$

m/e 238[M]$^{+\cdot}$

m/e 238[M]$^{+\cdot}$ →	m/e 220	-18	(H_2O)
m/e 220 →	m/e 205	-15	($CH_3\cdot$)
m/e 205 →	m/e 187	-18	(H_2O)
→	m/e 177	-28	(CO)
m/e 220 →	m/e 177	-43	($C_3H_7\cdot$)
→	m/e 163	-57	($C_4H_9\cdot$)
→	m/e 150	-70	(C_5H_{10}, C_4H_6O)
m/e 238[M]$^{+\cdot}$ →	m/e 153	-85	($C_5H_9O\cdot$)
m/e 153 →	m/e 135	-18	(H_2O)
m/e 238[M]$^{+\cdot}$ →	m/e 152	-86	($C_5H_{10}O$)
m/e 152 →	m/e 134	-18	(H_2O)
m/e 134 →	m/e 119	-15	($CH_3\cdot$)
→	m/e 91	-43	($C_3H_7\cdot$)

m/e 238[M]$^{+\cdot}$	m/e 154	-84	(C_5H_8O)
m/e 154	m/e 111	-43	($C_3H_7\cdot$)
m/e 111	m/e 93	-18	(H_2O)
m/e 93	m/e 91	-2	(2H·)
m/e 111	m/e 109	-2	(2H·)
m/e 154	m/e 136	-18	(H_2O)
m/e 136	m/e 121	-15	($CH_3\cdot$)
	m/e 93	-43	($C_3H_7\cdot$)

m/e 238[M]$^{+\cdot}$	m/e 111	-127	($C_8H_{14}OH\cdot$)
m/e 111	m/e 109	-2	(2H·)
m/e 109	m/e 107	-2	(2H·)
m/e 107	m/e 79	-28	(CO)

m/e 238[M]$^{+\cdot}$	m/e 71	-167	($C_{11}H_{18}OH\cdot$)
m/e 71	m/e 43	-28	(C_2H_4)

VIII. EXPERIMENTAL

1. Instruments, Equipment and Materials

The following instruments, equipment and materials were used:

Instruments:

F and M Model 500 Gas Chromatograph, thermal conductivity detector

Beckman DK-2A Ratio Recording Spectrophotometer

Beckman IR_{10} Spectrophotometer

Varian A-60D Analytical NMR Spectrometer

A.E.I. MS12 Mass Spectrometer coupled to an Aerograph Model 1200 Hy-Fi III Gas Chromatograph

A.E.I. MS9 Mass Spectrometer

Equipment:

Se-30 column: 10% Silicone gum rubber on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

Lac-728 column: 10% Diethylene glycol succinate on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

Carbowax 20M column: 10% Polyethylene glycol on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

Apiezon L column: 10% Apiezon L on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

Steam distillation apparatus, locally constructed

Materials:

Devil's Club root bark (Oplopanax horridum) was purchased from Dominion Herb Distributers, Montreal.

The following terpenes were obtained commercially: methyl heptenone* (K & K Laboratories Inc.), fenchone and pulegone (City Chemical Corporation), d-limonene and geraniol (Matheson Coleman and Bell), terpinyl acetate and safrole (Fritzsche Brothers of Canada Ltd.), eugenol (Shawinigan), and l-bornyl acetate (J.T. Baker Chemical Co.).

Methyl eugenol was prepared by E. Mah in this department.

These reference compounds were checked for purity on the Lac-728 column and purified by gas-liquid chromatography when necessary.

Oplopanone was kindly supplied by Shionogi Research Laboratory, Shionogi and Co. Ltd., Fukushima-ku, Osaka, Japan.

2. Preparation of Steam-Volatile Oil

The essential oil was prepared by steam distillation as designed in Remington, p. 174 (258), with modification. One-half kilogram of coarsely powdered root bark was covered with distilled water in a five liter round bottomed flask which was heated to about 90°. Steam was introduced into the flask. Distillation continued for 4 hours. The distillate which contained floating yellow oil droplets was saturated with sodium chloride (259), and extracted

*2-Methyl hepten-(2)-one(6)

with 250 ml portions of ether. Extraction was repeated until complete exhaustion was effected.

The combined ether extract was concentrated to a small volume by a laboratory rotatory vacuum evaporator, and treated with 5% sodium bicarbonate solution to separate acids from the oil. The non-acid portion of the oil was washed with saturated solution of sodium chloride, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The alkaline aqueous extract was acidified with hydrochloric acid, extracted with ether, and treated in the same way as the non-acid portion.

Five successive distillations were carried out in the same way. The essential oil of each distillation was stored in a dry clean tight container after flushing with nitrogen gas (260), wrapped in aluminium foil, and refrigerated.

The average yield was 6.85 gm (1.37% w/w) of a yellow colored oil. The non-acid portion, which represented 60% w/w of the oil, was investigated.

3. Separation and Collection of Components of Oil

Gas-liquid chromatography:

The instrument used was F and M model 500 gas chromatograph equipped with thermal conductivity detector, and employing helium as the carrier gas. The three columns which satisfactorily separated components of the oil were: Carbowax 20M, Se-30, and Lac-728.

The programmed temperature operation technique was

followed. Variations in: the column temperature, attenuation, and volume of injected sample were made until a satisfactory resolution was obtained. Conditions for optimum resolution using Se-30 column were:

Column temperature: programmed
Initial temperature: 100°C
Final temperature: 175°C "held"
Program rate: 5.6°C/min
Injection temperature: 215°C
Detection temperature: 250°C
Carrier gas: helium
Carrier gas flow rate: at detector exit 60 ml/min.
at reference exit 25 ml/min.
Bridge current: 150 mA
Attenuation: X2
Sample size: 5 µl

Conditions for optimum resolution using Lac-728 column were:

Column temperature: programmed
Initial temperature: 75°C
Final temperature: 170°C "held"
Program rate: 5.6°C/min.
Injection temperature: 215°C
Detection temperature: 300°C
Carrier gas: helium
Carrier gas flow rate: at detector exit 60 ml/min.
at reference exit 25 ml/min.
Bridge current: 150 mA

Attenuation: X2

Sample size: 5 µl

A volume of 5 µl of the essential oil from each of the five distillation batches was chromatographed on Se-30 and Lac-728 columns. The five chromatograms obtained from each column were identical, almost superimposable. So it was decided to mix all the samples together. The oil was then filtered to remove some fine white wax-like particles.

Thirty three measurable peaks were observed, using the columns C-20M, Se-30 and Lac-728. The best resolved peaks were observed when Lac-728 was used.

The retention time, in minutes, was calculated by measuring the distance on the chromatogram from the point corresponding to the beginning of the run to the centre of the peak (in mm) and multiplying by its equivalence in time after calibrating the chromatogram paper.

The percentage of each fraction of the oil was calculated from the chromatographic peak areas. The peak area was calculated by multiplying the height of the peak by the base at half height (261).

Augmentation of peaks:

For preliminary identification of the constituents of the oil, different known terpenes were injected. A volume of 4 µl of the oil plus 1 µl of the known terpene was injected into the Lac-728 column and the augmented peak was observed. The same procedure was repeated using Se-30 column. Eleven peaks were augmented on each column. Peak

no. 19 (Lac-728) was augmented by both terpinyl acetate and pulegone.

Collection of fractions:

The fractions were collected from the Lac-728 column. One end of an open ended melting point capillary tube was inserted through a hole in a rubber septum installed at the exit port of the chromatograph, and the other end was placed in an ice cooled bottle. The first seven fractions were very difficult to collect because of their high volatility. Fraction 2 although present in relatively high quantity, about 25%, could not be collected in this manner. It condensed and evaporated before reaching the bottle. A trap for collection of gas chromatograph fractions (Cat. 66 LG-8611 Labglass Inc. Vineland, N.J.) was used.

To obtain reasonable quantities of the fractions large volumes, approximately 50 µl, of the oil were injected. This resulted in overlapping of some peaks. The fractions were reinjected in smaller quantities for purification. To obtain the purest possible samples the fractions were collected by heart cutting of each peak (262). A further test for purity was performed by injecting the collected fractions into another column (Se-30) when possible. Only Fractions 2, 22, 25, 26, 31 and 33 were collected.

The collected fractions were subjected to infrared, ultraviolet and high-resolution mass spectrometric analyses. An NMR analysis was done when enough samples were available.

The oil was subjected to mass spectrometric analysis

by using gas chromatograph coupled to mass spectrometer.

4. Instrumental Data

The infrared spectra were recorded on Beckman IR10 spectrophotometer, using the thin film technique. The spectrum of Fraction 1 was recorded using chloroform solution in microcell and solvent compensation. The spectrophotometer was calibration checked by standard polystyrene film.

The ultraviolet spectra were recorded on Beckman DK-2A spectrophotometer using ethanol 95% as solvent. As the path length was 1 cm for all the spectra the molecular absorbance coefficient was calculated using the following formula (263):

$$\varepsilon = \frac{\text{absorbance} \times 100 \times \text{molecular weight}}{\text{Weight of compound in mg in 100 ml of solution}}$$

The nuclear magnetic resonance spectra were recorded at 60 Mc and tetramethylsilane was employed as an internal reference. The normal sweepwidth for the chart was 500 cps. The samples were dissolved in deuterated chloroform. Peaks ($\delta \simeq 7.25$ ppm) were associated with traces of $CHCl_3$ in $CDCl_3$ solvent.

The mass spectra of the collected fractions were recorded on A.E.I. MS9 mass spectrometer with heated inlet temperature 185°C, at 70 eV, and ion source temperature 100°C, unless otherwise mentioned.

The mass spectra of all fractions were recorded on A.E.I. MS12 mass spectrometer coupled to Aerograph model 1200

Hy-Fi III gas chromatograph under the same gas chromatographic conditions used in separation of the components. Volumes of 2 µl were injected into the same Lac-728 column. The mass spectra were recorded in the course of four chromatographic runs at 70 eV, and ion source temperature 100°C.

The backgrounds were subtracted from the spectra obtained from MS12 mass spectrometer. There were large peaks at m/e 18, 28 and several other small peaks.

All mass spectra are quoted in terms of relative abundance, with the most intense peak, "Base Peak", being taken as 100%.

IX. SUMMARY

The volatile oil of Devil's Club root bark obtained by steam distillation amounts to about 1.4% (of the dry weight) of which 60% (w/w) is non-acid.

The six compounds identified in this investigation (diethyl ether, ethanol, n-butyraldehyde, octanal, 2-nonanone and probably δ-cadinene) represent approximately 38% of the oil.

Three sesquiterpene hydrocarbons ($C_{15}H_{24}$) represent about 4.8%, and three oxygenated sesquiterpenes ($C_{15}H_{24}O$, $C_{15}H_{24}O_2$ and $C_{15}H_{26}O_2$) represent 12.1% of the oil.

Oplopanone ($C_{15}H_{26}O_2$) was not detected in the steam distilled oil.

It is probable that the steam distillation at atmospheric pressure might have caused some changes in the composition of the oil. The conditions of the gas-liquid chromatographic separation also might have caused some changes in the structure of the components.

The compounds identified frequently occur in various plant materials with the exception of ethanol.

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